

**PREHARVEST AFLATOXIN IN MAIZE GENOTYPES UNDER  
INOCULATION WITH *ASPERGILLUS FLAVUS***

A Thesis

by

KERRY L. MAYFIELD

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2006

Major Subject: Plant Breeding

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## ABSTRACT

Preharvest Aflatoxin in Maize Genotypes Under Inoculation

with *Aspergillus flavus*. (December 2006).

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Chair of Advisory Committee: Dr. F. Javier Betrán

Pre-harvest aflatoxin contamination is a major limitation to maize production in Texas and the southern United States, causing major economic loss and severe health problems worldwide. Screening for resistance to aflatoxin accumulation is commonly conducted through inoculation with a highly concentrated solution of *Aspergillus flavus* FR: Link spores, a naturally occurring fungus which infects maize and produces a toxic metabolite (aflatoxin) to humans and animals consuming the grain. No commercial hybrids exist with full resistance to aflatoxin accumulation; however, sources exist to reduce susceptibility. These sources commonly lack good agronomic characteristics for use in commercial hybrids. Exotic germplasm with favorable traits for reduced aflatoxin accumulations are introgressed with temperate and locally adapted lines. This program utilizes only one isolate of *A. flavus* even though many isolates exist in the environment. The objectives of this thesis are i) to evaluate the progress of the Maize Breeding and Genetics Program's accomplishments of breeding maize for the reduction in susceptibility of aflatoxin accumulation in yellow inbreds through analysis of hybrid and inbred *per se* trials and ii) to determine whether interaction exists between genetically-different isolates of *A. flavus* and several genotypes of maize. Response to aflatoxin

accumulation for hybrids and inbreds was measured at up to three environments across Texas. Significant differences were detected for most years and environments. Maize lines CML285, CML288, CML323, CML325, CML326, CML338, Tx601y and lines derived from Population 69 and from Tx772 crosses in hybrid combinations tended to accumulate less aflatoxin than commercial hybrid checks. Significant differences were detected at each environment aflatoxin accumulation was measured for inbred lines *per se*. Inbreds Tx772, Tx601y, CML289, CML294, CML323 and derived lines from Population 69 show reduced aflatoxin accumulations. Interaction between genetically different isolates of *A. flavus* and several genotypes of maize were not detected in hybrid or inbred trials at two or three environments, across locations and across years. Introgression of exotic germplasm into locally adapted germplasm has improved agronomic characteristics for use in the Southern U.S. and brought sources for decreased aflatoxin accumulation.

## **DEDICATION**

This thesis is dedicated to all those who have helped me and guided me along the way.

To my parents for allowing me to make my own decisions as I was aging regarding my life and my education.

To my son, who doesn't know any better now, but will realize hard work and determination will take him a long way.

Mostly, to my wife who had confidence in me when I didn't.

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Many thanks are necessary for all of the personnel who were part of these projects over the many years data were collected. Without their help and support, these experiments would not have the quality and the meaning that are presented.

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## **CHAPTER I**

### **INTRODUCTION**

Maize (*Zea mays* L.) became the most produced cereal crop in the world, surpassing wheat and rice in 2001 in terms of amount of grain produced (FAO, 2006). The U.S. is the largest producer of maize in the world, followed by China, Brazil, México and Argentina (FAO, 2006). The United States maize production is mainly concentrated in the Midwest states of Nebraska, Iowa and Illinois (NCGA, 2006a). Maize production in 2005 in the south was mainly produced in Texas with other Southern States producing less than one million acres each (NCGA, 2006b). Maize production in Texas during 2005 was an estimated 2,050,000 acres, with approximately 96% being harvested for either grain or silage (NASS, 2006). Country wide during 2005, 78 million acres of maize was planted with a harvest of 97%.

Uses of maize have expanded from food and feed stuffs to uses as fuel, sweeteners and starch products (TCPB, 2006). The uses of maize beyond what is now being used is continuing, with new uses including nutraceuticals, enzymes, pharmaceuticals and degradable plastics (NCGA, 2006b). Growing markets such as this could be boom for maize producers across the world as tougher restrictions are placed on petroleum based fuels. However, contamination of aflatoxin in maize still affects non food or feed directed industries. Use of maize in ethanol production is limited to maize with either low or no accumulations of aflatoxin (Robertson, 2005). During the

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This thesis follows the style of Crop Science.

distillation process, aflatoxins are not broken down, however; the produced alcohol contains no aflatoxins (Murthy et al., 2005; Lillehoj and Lagoda, 1979). Millers however may refuse the contaminated maize, because they cannot use the distillers grains as food or feedstuffs due to a three to four fold increase in concentration of aflatoxin (Hurburgh, 2005).

*Aspergillus flavus* is a naturally occurring fungus which infects maize and produces a toxin (aflatoxin) which is toxic to humans and animals consuming the grain. Pre-harvest aflatoxin contamination is a major limitation to maize production in Texas and the southern United States and to other areas of the world where seasonally high temperature and drought occur during the growing season, causing major economic loss (\$85-100 million lost in 1998) and severe health problems worldwide (80 Kenyans died from aflatoxin poisoning in 2004). Screening for resistance to aflatoxin accumulation is commonly conducted through inoculation with a highly concentrated solution of *Aspergillus flavus* FR:Link spores.

Aflatoxin in the United States is regulated in terms of commerce and consumption. Concentrations of aflatoxin above 20 ng g<sup>-1</sup> are banned from interstate commerce, human and dairy consumption, while concentrations above 300 ng g<sup>-1</sup> are banned for use as beef cattle feed.

There are no commercial hybrids with full resistance to aflatoxin accumulation. Sources of germplasm are available that have shown to reduce aflatoxin accumulation in maize, however; these sources commonly lack good agronomic characteristics to use in commercial hybrids. The Texas A&M Maize Breeding and Genetics Program has been

actively breeding yellow, white and quality protein maize to reduce aflatoxin accumulation. Much of the breeding has involved introgressing material of exotic origins with favorable traits (hard kernel endosperm, good kernel integrity, long tight husk cover, etc) to reduce aflatoxin. These exotic materials are crossed with temperate and locally adapted lines, selected for traits previously listed, to develop inbreds with good agronomic traits and lowered susceptibility to aflatoxin accumulation.

Our program utilizes currently only one isolate of *A. flavus* (NRRL 3357) even though many isolates exist in the environment (Bayman and Cotty, 1991; Ramaswamy, 2002). Isolates of *A. flavus* vary in toxicity, ranging from atoxigenic (producing no aflatoxin) to toxic (range of toxin produced varies as well). Atoxigenic strains are being evaluated for beneficial uses as a way to control aflatoxin accumulation in cotton, maize and peanuts (Antilla and Cotty, 2005; Dorner, 2005). The variability present for this pathogen in the field has raised the question as to whether different isolates of *A. flavus* interact differently with different genotypes of maize.

The objectives of the research presented in this thesis are (1) to estimate the response of experimental yellow hybrids and inbreds to aflatoxin accumulation under inoculation with *A. flavus* and identify possible germplasm sources to reduce susceptibility to aflatoxin and (2) to determine if there is interaction between genetically-different isolates of *A. flavus* and several genotypes of maize.



## CHAPTER II

### REVIEW OF LITERATURE

#### Maize

##### *Maize Background*

Maize is thought to have originated in Central México, due to the common locations of close genetic relatives, teosinte and *Tripsacum* (Jugenheimer, 1976; Wilkes, 2004). México and Guatemala have the largest diversity of teosinte and *Tripsacum* in the world. Modern maize is decisively different from its ancestral relatives and is definitely a plant which cannot survive on its own (Jugenheimer, 1976; Wilkes, 2004). Modern maize has specialized ears which lack a mechanism for seed dispersal compared with that of *tripsacum*. Maize was believed to have been discovered and distributed to the rest of the world from the Americas when Spanish explorers in 1492 brought back grain that was reported as good tasting from Cuba (Jugenheimer, 1976; Wilkes, 2004).

##### *Maize Current*

Maize is the most important cereal grain in Texas, during crop year 2005, producers harvested an estimated yield of 210,900,000 bushels with an estimated value of \$527,250,000.00 (USD) (NASS, 2006). U.S. production for that same time period was 11,112,072,000 bushels of maize (NCGA, 2006a). Uses of maize include food for human use and feed for livestock and recently have moved into new industries including starches, sweeteners, alcohols and plastics (TCPB, 2006)

Maize has had much advancement over the 20<sup>th</sup> century, going from open pollinated varieties to high yielding hybrids. More recently, biotechnology has brought resistance to selected herbicides and insects. These advancements have not been able to decrease, on large scale the contamination which occurs from fungi, specifically *Aspergillus flavus* (aflatoxins).

### **Aflatoxin**

#### *Aspergillus flavus Background*

*Aspergillus flavus* can produce aflatoxin, a toxic metabolite that has been shown to cause toxicosis in animals and hepatic cancer in humans (Castegnaro and McGregor, 1998). This known toxicity is regulated by many of the world's governments for commerce and consumption of the affected grains. *Aspergillus flavus* has the capability to produce aflatoxin in maize, cotton, sorghum, peanuts, tree nuts and groundnuts.

The best way to get accommodate aflatoxin resistance in maize is through the host plant resistance (Munkvold, 2003; Moreno and Kang, 1999). Transgenic hybrids resistant to ear damaging insects, which may otherwise give an open entry for the fungus, may be helpful in lowering incidence of aflatoxin (Munkvold, 2003).

Environmental conditions influence aflatoxin production; typically aflatoxins occur when high ambient temperatures and low soil moisture conditions are present. Lillehoj et al. (1975) found that aflatoxin occurrence and accumulation varied by location. *Aspergillus flavus* is a relatively weak pathogen; therefore non-inoculated tests are variable in the total amounts of aflatoxin accumulated and variable for its location in the field. Inoculating maize with the fungi helps remove natural variation of the fungus.

Scott and Zummo (1988) compared different inoculation techniques for *A. flavus*, utilizing a knife dipped in a suspension of *A. flavus* spores to cut through the husks into the kernels, spraying the silks with a suspension of spores and using the needle in the silk channel method to introduce the spores to the ear without damaging the kernels. A lower total accumulation with an increase in variability was identified for total aflatoxin produced using the needle in the silk channel method and spraying the silks with a suspension of spores than the wounding method. The increase in variability may be attributed to lower mean of aflatoxin accumulation attained by utilizing non-injurious methods of inoculation (Scott and Zummo, 1988). Even with lower accumulations, non-injury methods of inoculation proved to discriminate differences and provided for possible mechanical means of resistance, rather than only chemical means.

#### *Aflatoxin Resistance—Traits and Germplasm*

Several sources of resistance to aflatoxin accumulation are currently available to breeding programs. Inbred line Tx772 was released for its lowered susceptibility for aflatoxin accumulation. This line has traits such as long husks and orange flinty kernels, which may aid in the lowered susceptibility (Betran et al., 2002). Inbred Mp313 (Scott and Zummo, 1990), inbred Mp420 (Scott and Zummo, 1992), inbred Mp715 (Williams and Windham, 2001), inbred Mp717 (Williams and Windham, 2006), Population MAS: gk (McMillan et al., 1993), and inbred Tx807 (Moore et al., 2005 and Betran et al., 2003) constitute the majority of the sources adapted for southern environments of known lowered susceptibility for aflatoxin accumulation that are available to breeders. The majority of the previously stated inbred lines lack good agronomic characteristics. White

and Rocheford (2005) have been utilizing some of the above sources of germplasm coupled with molecular assisted backcrossing to transfer regions into commercially productive Corn Belt inbred lines.

Introgression of exotic germplasm with novel alleles and traits into U.S. breeding programs is one approach to reduce aflatoxin contamination (Betran et al., 2005). Exotic subtropical/tropical material has shown reduced accumulations of aflatoxin than temperate material (Betran et al., 2005). Traits such as ear rot, husk cover and insect damage have been shown to have a significant correlation for reducing aflatoxin accumulations (Betran et al., 2005).

Kernel integrity should be included in the list of traits which are believed to have an impact on aflatoxin accumulations in maize. Odvody et al. (1997) identified silk cut as one method of loss of kernel integrity and allowing fungi direct access to the kernel. Silk cut is variable but has occurred in vulnerable hybrids with loose, short husks exposing the tip of the ear and those hybrids susceptible to drought stress with high ambient temperatures (Odvody et al., 1997).

Although significant differences were not shown, Barry et al. (1986) concluded that husk tightness has an impact on preharvest aflatoxin by keeping the integrity of kernels intact. Barry et al. (1992) concluded insect damage is associated with aflatoxin accumulation in maize. Environmental conditions can become right for southwestern corn borer to have an increased effect on aflatoxin accumulations in maize (Williams et al., 2002). The use of hybrids genetically modified to resist insect damage could be a source of lowered susceptibility to aflatoxin accumulations.

Betran and Isakeit (2004) tested whether early maturing hybrids could benefit late season drought prone areas and found environmental adaptation had a larger effect on lowering susceptibility than early maturity. Husk cover was correlated with aflatoxin contamination and no injurious insect activity was observed.

Chen et al. (1998) identified resistance for aflatoxin contamination for seven genotypes to be associated with high levels of a 14-kDa protein with in kernels. This 14-kDa protein is a trypsin inhibitor possibly related to the opaque-2 gene.

Chemical resistance within the kernel would circumvent any loss of kernel integrity by using wounding inoculation techniques (Campbell and White, 1995). Carotenoids have shown to strongly inhibit aflatoxin B<sub>1</sub> production (Norton, 1997). Norton (1997) feels many current maize lines have carotenoid levels high enough to help prevent aflatoxin accumulation; however, Wicklow et al. (1998) reported that there is not strong evidence in the literature for or against this theory. The isolate being considered may or, may not, be sensitive to carotenoids due to the natural variation of the organism, which is the case with *A. flavus* NRRL3357 (Wicklow et al., 1998).

#### *Variation of Aspergillus flavus*

Communities of *A. flavus* are variable between continents, across continents, with in fields and with in local areas (Cotty and Cardwell, 1999; Cotty, 1997; Ramaswamy, 2002). *A. flavus* is classified into different strains, L (large sclerotia) and S (small sclerotia) (Cotty, 1997), with the S strains typically producing more aflatoxin than the L strains. Larger percent of S strains (52%) versus L strains (48%) were isolated in a maize field in South Texas (Ramaswamy, 2002).

*Aspergillus flavus* varies within the environment in toxicity production, ranging from atoxigenic to isolates which produce large amounts of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> aflatoxins (Cotty, 1997). Many breeding programs utilize only one isolate of *A. flavus* for inoculation of aflatoxin screening tests (Scott and Zummo, 1988; Norton, 1997; Barry et al., 1992; Lillehoj et al., 1975; Windham and Williams, 2002; Betran et al., 2002), while others use a multiple isolate cocktail (Campbell and White, 1995; Naidoo et al., 2002). No previous research was found determining differences in contamination levels of different isolates with different genotypes of maize in the field.

Naidoo et al. (2002) considered multiple (more than two) locations are necessary to identify genotypes superior to preventing the accumulation of aflatoxins in maize, due to concerns of variability of the fungi and aflatoxin production.

#### *Aflatoxin Control—Resistance and Bio-control*

Development of host resistance to aflatoxin accumulations would be the best solution to aflatoxin accumulation in maize (Barry et al., 1992; Windham and Williams 2002); however, Widstrom et al. (1984) identified the expense in dollars and labor needed to identify resistant genotypes to be extremely high, partially due to an inefficient screening method and high costs of the tests. Marker assisted selection can be utilized to effectively select for aflatoxin resistant material in germplasm with identified QTL. Marker assisted selection can increase effectiveness of time due to the lowly heritable trait (aflatoxin resistance) (Busboom and White, 2004) and potentially allow for selection with out inoculation and at multiple environments (Robertson et. al., 2005). The use of markers in any breeding program has positives and negatives, depending on

resources available. Morris et al. (2003) identified marker assisted selection as being more costly, however, consumed less time than utilizing only phenotypic selection.

Atoxigenic strains of *A. flavus* are currently being used as a biocontrol for aflatoxin accumulations in cotton, peanut and maize (Dorner, 2005; Cotty, 1990).

Atoxigenic strains are distributed in production fields to compete with the toxigenic strains for colonization. This competition ultimately leads to a decrease in aflatoxin accumulations.

## CHAPTER III

### AFLATOXIN EVALUATION OF MAIZE HYBRIDS

#### Introduction

##### *Background*

Aflatoxin was discovered to be a potential health problem in the mid 1900s, affecting crops such as maize, cotton, ground nuts and tree nuts. The use of host plant resistance has been postulated as the best control of aflatoxin in maize (Munkvold, 2003). Unfortunately, no commercial hybrids with complete resistance to aflatoxin accumulation are available to maize producers. However, there are sources to reduce susceptibility (Scott and Zummo, 1990, Scott and Zummo, 1992, Williams and Windham, 2001, Williams and Windham, 2006, McMillan et al., 1993, Moore et al., 2005 and Betran et al., 2003). These sources lower susceptibility to aflatoxin, but they lack good agronomic characteristics for use in commercial hybrids. Making inbred selections based on the response of their hybrids to aflatoxin accumulation can be a slow process. The use of favorable traits associated with reducing aflatoxin accumulation (husk cover, husk tightness, kernel integrity, grain texture, e.g.) may increase genetic gain.

Testing for presence of the toxin is also a bottleneck which must be overcome to proceed forward for identifying resistance. *Aspergillus flavus*' variable nature (within testing areas and between testing areas requires multiple replications and environments. Inoculation methods vary and can also contribute to aflatoxin accumulations. The costs



incurred in quantifying aflatoxin are high enough to limit the number of germplasm screened for resistance (Widstrom et al., 1984).

### *Current Actions*

The Texas A&M Maize Breeding and Genetics Program has been actively breeding yellow, white and quality protein maize to reduce aflatoxin accumulation. Much of the breeding has involved introgressing material of exotic origins with before mentioned favorable traits to reduce aflatoxin (hard kernel endosperm, good kernel integrity, long tight husk cover, etc) (Betran et al., 2006b). These exotic materials are crossed with temperate and locally adapted lines, selected for favorable traits for reducing aflatoxin accumulation, to have an inbred with good agronomic traits (maturity and structure) and lowered susceptibility to aflatoxin accumulation (Betran et al., 2005).

The objective of this section is to present and discuss a multiyear multilocation evaluation of maize hybrids for response to aflatoxin under inoculation with *A. flavus* and the possibility of identifying possible sources of resistance that reduce susceptibility of aflatoxin.

## **Materials and Methods**

### *Germplasm*

Five years of yellow hybrid aflatoxin evaluations were conducted at two or three different Texas locations (Figure 3.1). Hybrids with diverse origins and genetic backgrounds were evaluated to estimate their effectiveness in reducing aflatoxin contamination and which phenotypic traits were associated with resistance. Most of the inbreds were developed from the introgression of exotic germplasm and selected for

those traits which may be related to the reduction of aflatoxin accumulation in maize. A complete listing of pedigrees of hybrids and inbreds can be found in Tables 3-1 to 3-28 in the results section.

#### *Field Evaluation of Aflatoxin*

An alpha lattice experimental design was used for all trials with three to nine replications. When 9 replications were used, groups of 3 replications were combined for quantification at each environment. Experimental units consisted of one or two row plots. Trials were planted in the spring, at or later than optimal planting time. Drought stress on trials was induced by either withholding irrigation at College Station and Weslaco or delaying planting at Corpus Christi. *Aspergillus flavus* isolate NRRL3357 was used for all trials. A conidial suspension containing  $3 \times 10^7$  conidia of *A. flavus* in 3 mL distilled water was injected 6 to 10 d after mid silk by the silk channel inoculation technique (Zummo and Scott, 1989) or by placing colonized kernels in the row between plots (Odyssey et al., 2000). Approximately 1kg of colonized maize kernels was applied per 200 feet of row length when using the colonized kernel method of inoculation.

#### *Field Measurements*

Visual ratings were taken in the field for grain yield, ear aspect, grain texture, husk tightness, husk cover, lodging, plant aspect, visual *Aspergillus flavus* colonization, insect damage rating, ear rot, plant appearance, desirability, and kernel integrity. Traits measured prior to harvest include plant height, ear height and early vigor. Grain moisture, test weight, grain yield, ear yield and aflatoxin were measured after harvest in the lab.

Flowering was measured to assist in timing of inoculation of *A. flavus* and was recorded as number of days from planting to 50% of the plants showing silks for silking date or shedding pollen for anthesis date. Anthesis silking interval was calculated by subtracting silking date from anthesis date. Grain yield was measured as plot weight and transformed to  $\text{Mg ha}^{-1}$ . In those experiments where grain yield was not measured a subjective rating was recorded (scale of 1=good yield to 5=poor yield). Ear aspect was rated from 1=good ear aspect to 5=bad ear aspect ear. Grain texture was recorded using a rating scale of from 1=hard, completely rounded kernel to 5=soft, distinct dent. Husk tightness was recorded using a scale from 1=tight husk leaves around the ear to 5=husk leaves loose and allowing the ear to be exposed. Husk cover was rated using a scale from 1=long husk covering the entire length of the ear to 5=short husk with ear protruding and kernels exposed. Stalk and root lodging were measured as percentage of plants per plot affected by broken stalks below the ear bearing node or by leaning stalks more than  $30^\circ$  from the vertical, respectively. Lodging was also recorded using rating from 1=all plants standing to 5=majority of plants lodged. Plant aspect was recorded using ratings from 1=good conformity, low ear placement to 5=poor conformity, high ear placement. Visual *A. flavus* rating was recorded as 1=no colonization on ear to 5=ear colonized heavily. Plant height and ear height were measured as the distance in cm from the ground to the top of the tassel and point of attachment of the ear shank, respectively. Insect damage ratings were recorded from 1=no damage or channeling to 5=heavy damage or channeling. Early vigor was rated as 1= good early vigor to 5= low early vigor. Ear rot was recorded using a rating from 1=no ears rotted to 5=ears mostly rotted

and unshellable. Plant appearance was recorded as rating from 1=dark green erect leaves to 5=light green flat leaves. Kernel integrity was recorded as ratings from 1=all ears with out split kernels or damage by insect to 5=most of the ears with split and/or damaged kernels.

Grain yield was measured as whole plot weights and converted to  $\text{Mg ha}^{-1}$ . Plots were either hand harvested or combine harvested after the inoculated plants were hand harvested. The inoculated ears were shelled, weighed and added back to the combine weight. Grain moisture and test weight measured on combine mounted equipment. Ear yield is calculated by measuring the bulk shelled maize of the plot and dividing the weight by the number of ears harvested and expressed in grams.

#### *Aflatoxin Quantification*

All of the plants in an experimental unit were harvested in trials inoculated using the colonized kernel method while only inoculated plants were harvested in trials inoculated using the silk channel method. Samples were shelled with a maize sheller and the grain was ground using a Romer Mill (Romer Labs, Union, MO). Quantification of aflatoxin was conducted in 50 g subsamples from each plot with monoclonal antibody affinity columns and fluorescence determination by the Vicam Aflatest (Watertown, MA).

*Statistical Analysis*

Single location data were analyzed as Randomized Complete Block Design and Alpha Lattice incomplete block, using SAS Proc GLM and Proc Mixed and using REMLTool with and without spatial analysis. Aflatoxin concentration was log transformed (base 10) to standardize variances and reported as a geometric mean (antilogarithm). Aflatoxin concentration was expressed in nanograms per gram ( $\text{ng g}^{-1}$ ). Means obtained with the most efficient analysis (i.e., having the lowest error) were reported. In some experiments some entries in different environments of the same year were different. In these cases the analysis across locations was conducted with only common entries across environments. Best Linear Unbiased Predictors (BLUPs) were estimated using SAS Proc GLM and Proc Mixed procedures, respectively.

Additive Main Effects and Multiplicative Interaction (AMMI) analysis of aflatoxin in inbreds and hybrids at different environments was carried out to assess the relationship among genotypes and environments using Biplot v1.1 (Dr. E.P. Smith, Virginia Tech; <http://www.stat.vt.edu/facstaff/epsmith.html>).

## Results

The mean aflatoxin accumulation at College Station in 1999 was  $114 \text{ ng g}^{-1}$  with a range of  $11.5 \text{ ng g}^{-1}$  to  $269.2 \text{ ng g}^{-1}$  (Table 3-1). Significant differences were detected for all traits measured. Line Tx772, crossed with temperate testers was part of the two hybrids less susceptible to aflatoxin accumulation. The third less susceptible hybrid for aflatoxin accumulation was CML291 (a tropical line from CIMMYT) crossed by temperate tester LH210.

Hybrid Tx772 x FR1130 had the second lowest rating for grain texture (1.6), the lowest rating for husk cover (1.5), and the second lowest rating for *A. flavus* colonization ratings (1.3) (Table 1). Hybrid Tx772/(FR2128xFR1130) had the second lowest rating for grain yield (1.9) and the lowest rating for grain texture (1.4) (Table 1). Hybrid CML291/LH210 had a low rating for *A. flavus* colonization (1.6) (Table 1), however; did not exhibit desirable expression for husk cover or grain texture. In general, tropical and subtropical lines crossed with temperate testers tend to show traits related to reduced aflatoxin accumulation (tight husk cover, longer husks and hard endosperm texture).

Table 3-1. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at College Station, TX in 1999.

Entry	Hybrid	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	GY	EA	TXT	HT	HC	LD	PA	AFR
			ng g <sup>-1</sup>	d	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5
1	LB31/LH210	16	269.2 a <sup>§</sup>	78.5	3.8	3.9	4.4	4.3	3.5	2.3	4.3	1.9
2	MAS gk/LH210	13	218.8 a,b	77.8	3.3	3.5	4.1	2.9	1.5	1.9	2.6	2.3
3	Tx714/LH210	12	182.0 a,b	76.8	2.1	2.3	3.9	3.5	3.0	1.9	2.5	1.5
4	FR2128 (B73)/LH210	15	229.1 a,b	77.8	2.3	1.9	2.8	3.0	2.4	2.0	2.9	1.5
5	B97/LH210	10	144.5 a-c	76.3	3.5	3.4	4.6	4.3	3.4	2.4	3.5	3.0
6	CML326/LH210	4	70.8 b-d	78.0	1.4	1.4	2.0	3.1	3.1	1.6	2.0	1.5
7	CML291/LH210	3	25.1 d-f	80.3	2.1	2.0	2.3	3.1	3.1	1.0	1.9	1.6
8	CML337/LH210	11	93.3 a-c	78.3	2.3	2.5	4.3	3.8	3.1	1.9	2.0	2.3
9	SCB GCAC0#-63-2-2-2-1-3-B*4-B/LH210	7	87.1 a-c	75.3	2.8	2.5	2.1	3.0	3.3	2.1	3.1	1.3
10	CML193-B/LH210	9	97.7 a-c	78.3	2.4	2.6	4.1	2.9	2.4	1.5	2.3	2.3
11	Tx820/LH210	8	85.1 a-c	78.5	1.9	1.6	3.1	3.1	3.1	1.4	1.8	1.5
12	Tx772/FR1130	1	11.5 f	77.5	2.0	2.0	1.6	2.5	1.5	2.0	3.1	1.3
13	Tx714/FR2128	6	85.1 a-c	78.0	2.1	2.1	1.8	3.8	3.5	2.3	3.1	2.4
14	75-R001/(Tx807xTx811)	14	158.5 a,b	75.5	4.0	4.8	3.0	3.1	2.8	2.8	4.4	1.8
15	MP420/(Tx807xTx811)	5	47.9 c-e	78.5	2.9	2.9	2.0	2.1	1.8	1.6	2.4	2.3
16	Tx772/(FR2128xFR1130)	2	17.8 e,f	78.3	1.9	1.6	1.4	3.0	1.9	1.8	2.8	1.1
	Mean		114.0	77.7	2.5	2.6	3.0	3.2	2.7	1.9	2.8	1.8
	LSD(0.05) <sup>¶</sup>		.	2.1	1.0	0.9	0.7	0.8	1.3	0.9	1.1	1.19
	Genotype Sig.		**	**	**	**	**	**	*	**	**	*
	C.V.% <sup>#</sup>		18.9	1.6	23.7	21.4	15.3	15.2	28.6	28.3	23.9	39.6

\*, \*\* and \*\*\* Significant at .05, .01 and .001 levels, respectively

<sup>†</sup>Rank of genotypes by aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, GY: visual rating for grain yield (1=high grain yield to 5=low grain yield), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), HT: Husk Tightness (1=tight to 5=loose), HC: Husk Cover (1=long to 5=short), LD: Lodging (1=all plants standing to 5=all plants lodged), PA: Plant Aspect (1=desirable to 5=undesirable), AFR: visual rating for *Aspergillus flavus* Colonization (1= no colonization to 5=all ears colonized)

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

The mean aflatoxin accumulation for yellow hybrids at Weslaco, TX in 1999 was 385.4 ng g<sup>-1</sup> with a range of 97.66 ng g<sup>-1</sup> to 1027.07 ng g<sup>-1</sup> (Table 3-2). Significant differences for hybrids were detected for aflatoxin, anthesis date, silking date, ear aspect, grain texture, husk tightness, husk cover, plant aspect, plant height and ear height. No significant differences for hybrids were detected for anthesis silking interval and grain yield.

Line Tx772 crossed with a temperate tester (FR1130) was again the least susceptible hybrid for aflatoxin accumulation. The second less susceptible hybrid for aflatoxin accumulation was a temperate line (FR2128) crossed with temperate tester LH210. The third less susceptible hybrid for aflatoxin accumulation was line CML326 (a tropical line from CIMMYT) crossed with temperate tester LH210.

Hybrid Tx772 x FR1130 had the lowest rating for grain texture (1.0) and the second lowest rating for husk cover (1.9) (Table 2). Hybrid Tx772 x FR1130 did have the long anthesis silking interval (1.8 d) and the loosest husk tightness (4.0) (Table 3-2). Hybrid FR2128/LH210 had lower than average rating for grain texture (2.5) and lowest rating for husk cover (1.6) (Table 3-2). Hybrid CML326/LH210 tied for the second lowest rating for grain texture (1.5). Traits expressed (husk cover, grain texture and husk tightness) by these hybrids may have contributed to their low susceptibility.



Table 3-2. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at Weslaco, TX in 1999.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	MF	FF	ASI	GY	EA	TXT	HT	HC	PA
			ng g <sup>-1</sup>	d	d	d	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5
1	LB31/LH210	17	759.8 a,b <sup>¶</sup>	64.0	65.0	1.0	2.4	3.0	4.3	4.8	3.9	3.4
2	MAS gk/LH210	16	615.7 a-d	65.0	66.3	1.3	3.0	3.1	3.1	3.1	1.9	2.8
3	Tx714/LH210	6	252.4 d,e	65.0	66.0	1.0	3.0	3.0	3.1	4.5	3.3	2.6
4	FR2128/LH210	2	159.2 e,f	64.0	65.5	1.5	1.5	1.5	2.5	3.4	1.6	1.3
5	B97/LH210	10	351.2 b-e	63.3	64.0	0.8	3.4	3.5	5.0	3.8	3.0	2.0
6	CML 326/LH210	3	176.7 e,f	64.0	65.0	1.0	2.3	2.3	1.5	3.6	2.5	2.0
7	CML291/LH210	5	235.6 e,f	65.3	65.5	0.3	2.1	2.5	1.6	1.6	3.4	1.8
8	CML337/LH210	15	641.5 a-c	65.3	66.5	1.3	3.5	3.3	3.1	4.4	3.3	3.1
9	Dekalb 668	11	375.8 b-e	65.0	66.0	1.0	2.5	2.9	3.5	3.5	1.5	1.6
10	CML 193/LH210	14	385.0 b-e	65.0	65.5	0.5	2.9	3.0	3.5	3.6	2.6	2.5
11	Tx820/LH210	8	324.5 b-e	66.3	67.3	1.0	2.8	2.4	2.0	2.0	2.1	1.5
12	TX772/FR1130	1	97.7 f	65.0	66.8	1.8	2.1	2.1	1.0	4.0	1.9	3.5
13	Tx714/FR2128	9	344.2 b-e	66.0	67.5	1.5	1.6	1.8	1.5	3.9	2.6	2.6
14	LB31/(Tx807xTx811)	7	297.4 c-e	64.0	65.8	1.8	3.6	3.6	2.1	4.4	3.3	4.1
15	75-R001/(Tx807xTx811)	12	291.3 c-e	62.5	64.0	1.5	3.1	3.3	3.1	4.3	4.0	3.4
16	MP420/(Tx807xTx811)	13	378.6 b-e	65.8	67.0	1.3	3.0	3.3	1.8	2.5	2.0	2.3
17	Garst 8300 GLS IT	4	222.9 e,f	65.3	65.8	0.5	2.8	2.5	3.8	4.6	3.3	1.6
18	Pioneer 3223	18	1027.1 a	67.0	68.0	1.0	2.4	3.3	2.0	4.0	4.8	1.8
	Mean		385.4	64.9	66.0	1.1	2.7	2.8	2.7	3.7	2.8	2.4
	LSD <sup>§</sup>			0.8e	1.0			1.3	1.0	1.2	1.2	1.1
	Significance		***	**	**	NS	NS	**	**	**	**	**
	C.V.% <sup>#</sup>		11.3	0.8	0.9	61.8	31.3	29.1	20.3	20.7	25.0	26.6

\*\* Significant at .01 level, NS=Not significant at .05 level

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, MF: days from planting to mid-anthesis, FF: days from planting to silking, ASI: anthesis silking interval, GY: visual rating for grain yield (1=high grain yield, 5=low grain yield), EA: Ear Aspect (1= good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), HT: Husk Tightness (1=tight to 5=loose), HC: Husk Cover (1=long to 5=short), PA: Plant aspect (1=desirable to 5=undesirable)

<sup>¶</sup>Mean separations determined using the logarithmic transformation of the data

<sup>§</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

Fifteen hybrids were included across location analysis. The mean aflatoxin accumulation was  $194.6 \text{ ng g}^{-1}$  with a range of  $33.4 \text{ ng g}^{-1}$  to  $454.5 \text{ ng g}^{-1}$  (Table 3-3). No significant differences were detected for aflatoxin and silking date. Significant differences were detected for grain texture and husk cover.

Hybrid Tx772/FR1130 had the lowest numerical accumulation of aflatoxin ( $33.4 \text{ ng g}^{-1}$ ), tied for the lowest rating for grain texture, had the lowest rating for husk cover (1.3) and had above average silking date (72.1) (Table 3-3). Hybrid CML291/LH210 accumulated the second lowest numerical aflatoxin concentration ( $77.4 \text{ ng g}^{-1}$ ) and lower than average husk cover rating (1.9) (Table 3-3). Hybrid CML291/LH210 showed above average grain texture (3.3) rating and above average silking date (72.9 d) (Table 3-3). Both hybrids Tx772/FR1130 and Hybrid CML291/LH210 had relatively low aflatoxin accumulations in both College Station and Weslaco, giving low accumulation across locations. In contrast, hybrid MP420/Tx807xTx811 had small accumulations in College Station (Table 3-1) and larger accumulations in Weslaco (Table 3-2).

BLUPs are reported in Table 3-3 for aflatoxin, grain texture, husk cover and silking date across locations. Trends for rankings of means and BLUPs are the same with this set of data. The mean of the BLUP for the aflatoxin data is  $173.2 \text{ ng g}^{-1}$  with a range of  $62.3 \text{ ng g}^{-1}$  to  $301.0 \text{ ng g}^{-1}$ . The mean BLUP is  $21.4 \text{ ng g}^{-1}$  lower than that of the means across locations. BLUP in secondary traits across locations show the same response to rank and location around the mean as aflatoxin (Table 3-3).

Table 3-3. Means and statistics for aflatoxin and secondary traits across two locations in 1999.

Entry	Pedigree	Rank	AF <sup>†</sup>		TXT		HC		FF	
			Mean	BLUP <sup>‡</sup>	Mean	BLUP	Mean	BLUP	Mean	BLUP
			ng g <sup>-1</sup>	ng g <sup>-1</sup>	1 to 5		1 to 5		1 to 5	
1	LB31/LH210	13	454.5	301.0	3.7	3.5	4.3	4.2	71.8	71.9
2	MAS qk/LH210	12	367.3	264.8	1.7	1.8	3.6	3.6	72.0	72.0
3	Tx714-B/LH210	9	213.2	190.7	3.1	3.1	3.5	3.5	71.4	71.8
4	FR2128-B (B73)/LH210	7	190.0	177.9	2.0	2.1	2.6	2.6	71.6	71.9
5	B97/LH210	10	225.3	197.1	3.2	3.1	4.8	4.7	70.1	71.3
6	CML 326-B/LH210	4	112.2	129.4	2.8	2.8	1.8	1.8	71.5	71.8
7	CML291/LH210	2	77.4	103.5	3.3	3.2	1.9	2.0	72.9	72.3
8	CML337-B/LH210	11	244.1	206.9	3.2	3.1	3.7	3.7	72.4	72.1
10	CML 193-B/LH210	8	192.8	179.4	2.5	2.5	3.8	3.8	71.9	72.0
11	Tx820-B/LH210	5	167.1	164.6	2.6	2.6	2.6	2.6	72.9	72.3
12	TX772 x FR1130	1	33.4	62.3	1.7	1.8	1.3	1.4	72.1	72.0
13	Tx714 x FR2128	6	170.5	166.6	3.1	3.0	1.6	1.7	72.8	72.3
15	MP420/Tx807xTx811	3	81.8	106.9	1.9	2.1	1.6	1.6	72.6	72.2
	Mean		194.6	173.2	2.7	2.7	2.9	2.9	72.0	72.0
	LSD <sup>§</sup>		.		0.51		0.20		0.83	
	Genotype Sig.		NS		**		***		NS	
	Env. Sig.		***		***		***		***	
	G*E Sig.		**		NS		***		***	
	C.V.% <sup>#</sup>		0.73		3.49		10.89		4.68	

\*\* and \*\*\* Significant at .01 and .001 Levels, respectively, NS=Not Significant at .05 level

<sup>†</sup>Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint to 5=dent), HC=Husk Cover (1=long to 5=short), FF=days from planting to silking.

<sup>‡</sup>BLUP: Best Linear Unbiased Predictor

<sup>§</sup>Fisher's least significant difference

<sup>#</sup>C.V.%: Coefficient of Variation

Significant genotype\*environment interaction was detected for aflatoxin, husk cover and silking date. No significant genotype by environment interaction was detected for grain texture. Rank in aflatoxin accumulation at College Station and Weslaco changed significantly (Figure 3-1). Hybrid LB31/LH210 accumulated the most aflatoxin at both locations. Hybrid CML337/LH210 accumulated the second most at Weslaco, while it being one of the least susceptible hybrids in College Station. The significant interaction for these three traits shows that environment has an impact on the expression of traits for lowering the susceptibility of aflatoxin accumulation, given those traits are associated with reducing aflatoxin accumulation.

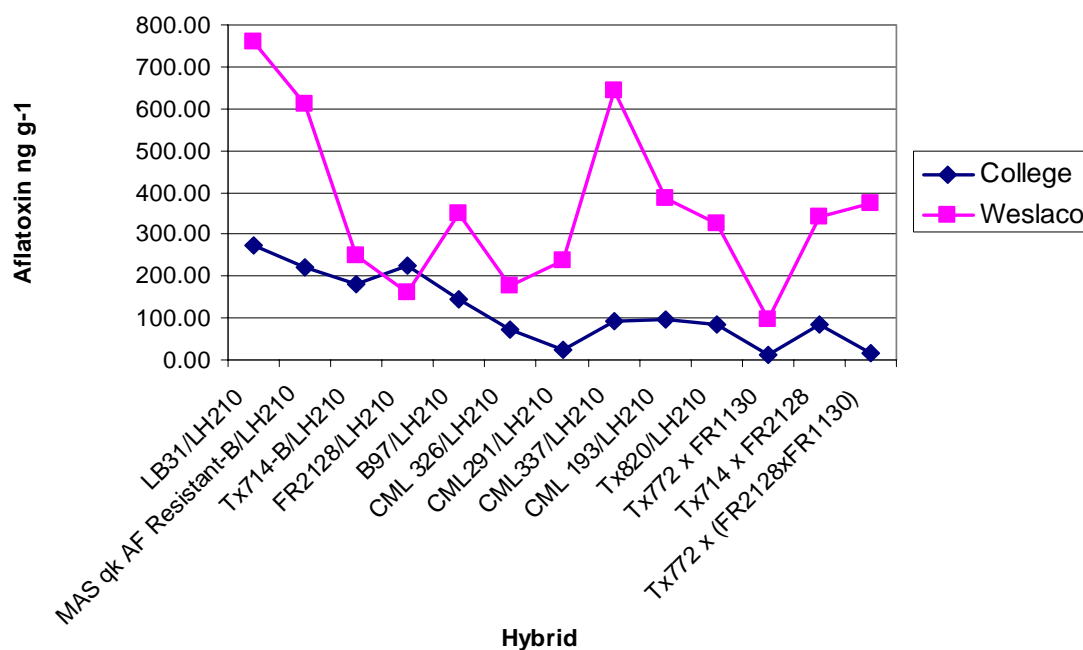


Figure 3-1. Mean aflatoxin accumulation per hybrid at two locations in 1999.

Population MAS gk is reported as having resistance to aflatoxin accumulation. However, crossed with temperate line LH210 accumulated some of the most aflatoxin in College Station and Weslaco. Differences exist between accumulations with line Tx772 and the two lines labeled as aflatoxin resistant, MAS gk and MP420, in these hybrid combinations at College Station and Weslaco.

*College Station 2000*

The mean aflatoxin accumulation for yellow hybrids at College Station in 2000 was  $95.9 \text{ ng g}^{-1}$  with a range of  $10.2 \text{ ng g}^{-1}$  to  $812.8 \text{ ng g}^{-1}$  (Table 3-4). Significant differences were detected for all traits measured. Lines Tx772 and MP715 were part of three hybrids less susceptible for aflatoxin. Tropical line CML285 (from CIMMYT), crossed with Tx772 was the least susceptible hybrid in this location. Line Tx772 and MP715, both released for lower susceptibility to aflatoxin accumulation had the second lowest accumulation of aflatoxin. The third less susceptible hybrid was a combination of Tx601y (a Southern U.S. line) and Mp715, both of which have shown lower susceptibility to aflatoxin.

Hybrid CML285/Tx772 had the lowest rating for grain yield (1.7), the lowest rating for ear rot (1.3), a tie for the lowest rating for ear aspect (1.5), a relatively dent grain texture (3.6) and a below average husk cover (2.3). Hybrid Tx772/Mp715 had the second longest silking date (83.3 d), a low ear rot rating (1.6), the hardest grain texture rating (1.3) and a tight husk cover (1.5). Hybrid Tx601Y/Mp715 had the shortest silking date (67.8 d), a below average rating for ear rot (1.6), the second lowest rating for grain texture (1.3) and a relatively long husk cover (1.5). Line Tx772 did not express some of

the traits previously expressed in other locations and years for lower susceptibility of aflatoxin accumulation, however; still did not accumulate large quantities of aflatoxin.

*Weslaco, TX 2000*

The mean aflatoxin accumulation for yellow hybrids at Weslaco, TX in 2000 was 317.7 ng g<sup>-1</sup> with a range of 10.7 ng g<sup>-1</sup> to 1819.7 ng g<sup>-1</sup> (Table 3-5). Significant differences were detected for each trait measured. Line Tx772 was a parent in two hybrids less susceptible for aflatoxin while the third less susceptible hybrid was a cross between subtropical line Tx601y and a Mp715.

Hybrid Tx772/Mp715 had a relatively long ASI (5.0 d) and a loose husk cover (4.5) rating but still had the lowest accumulation of aflatoxin (Table 3-5). Hybrid Tx772/CML326 had the second lowest grain texture rating (1.1), loose husk tightness rating (3.3) and higher than average husk cover rating (2.0) (Table 3-5). Tx601y/Mp715 had the second tightest husk tightness rating (1.9) and the third longest husk cover rating (1.5) (Table 3-5). Line Tx772 again did not express those traits previously expressed in other locations and years for lower susceptibility of aflatoxin accumulation, yet still was part of hybrids lower in susceptibility to aflatoxin accumulation.

Table 3-4. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at College Station, TX in 2000.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	FF date	GY 1 to 5	ER 1 to 5	EA 1 to 5	PA 1 to 5	TXT 1 to 5	HC 1 to 5	PH in	EH in
1	CML 326/Mp420	17	131.8a,b <sup>¶</sup>	79.5	3.6	2.4	3.6	3.1	1.4	1.5	78.1	28.2
2	TX772-/Mp420	7	22.9 b-f	81.0	3.6	2.3	3.9	4.2	1.3	1.0	77.7	31.5
3	CML285/Mp420	15	100.0 b-d	81.3	2.0	1.6	1.9	3.2	3.9	1.3	87.2	37.4
4	Tx601y/Mp420	9	25.1 b-f	85.8	2.6	1.5	3.0	4.4	2.8	1.0	88.6	37.9
5	FR2128(B73)/Mp420	16	114.8 a-c	80.5	2.0	1.6	2.1	2.0	2.8	1.0	76.1	30.8
6	TX772/CML326	5	22.4 d-f	78.5	2.8	2.4	2.8	3.6	0.8	1.8	67.8	24.0
7	CML285/CML326	8	22.9 b-f	81.3	2.1	1.4	2.3	2.6	1.4	2.0	79.6	29.5
8	Tx601y/CML326	11	28.2 b-f	84.5	2.1	1.5	2.0	2.8	1.8	1.5	85.6	30.5
9	FR2128 (B73)/CML326	10	25.7 c-f	81.5	3.3	1.7	3.3	2.3	1.7	2.3	72.7	27.3
10	CML285/TX772	1	10.2 f	86.5	1.7	1.3	1.5	3.4	3.6	2.3	85.3	40.9
11	Tx601y/TX772	6	22.4 b-f	83.8	2.3	1.6	2.1	4.2	1.6	2.0	81.7	30.8
12	FR2128(B73)/TX772	4	21.9 d-f	79.5	2.9	2.1	3.5	3.1	1.5	1.3	70.1	26.5
13	Tx601y/CML285	12	39.8 b-f	85.8	1.6	1.3	1.5	3.2	3.9	1.0	91.6	36.8
14	FR2128(B73)-B/CML285	13	69.2 b-e	80.5	1.9	1.5	2.1	1.8	3.1	2.0	82.6	33.3
15	FR2128(B73)/Tx601y	18	169.8 a,b	84.3	2.0	1.8	2.0	2.8	2.5	1.0	83.8	33.6
16	Tx601y/Mp715	3	21.4 c-f	67.8	3.4	1.8	4.0	4.2	3.9	2.3	84.3	42.1
17	FR2128(B73)/Mp715	14	75.9 b-f	83.8	2.5	1.8	3.0	2.7	2.9	3.0	85.7	37.2
18	TX772-/Mp715	2	11.7 e,f	83.3	2.5	1.6	2.6	3.6	1.3	1.5	81.4	37.8
19	DK668	19	169.8 b-d	79.5	2.1	2.0	2.3	1.8	4.0	1.3	70.2	25.0
20	P3223	20	812.8 a	78.8	2.9	4.3	4.0	2.3	3.5	3.0	73.9	27.0
	Mean		95.9	81.4	2.5	1.9	2.7	3.1	2.5	1.7	80.2	32.4
	LSD <sup>§</sup>			4.7	0.9	0.6	0.8	0.9	0.4	0.6	3.8	3.9
	Sig.		**	***	***	***	***	***	***	***	***	***
	C.V.% <sup>#</sup>		31.4	4.0	23.7	20.2	19.5	18.6	10.8	25.8	3.2	8.2

\*\* and \*\*\* Significant at .01 and .001 Levels, respectively

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, GY: visual rating for grain yield (1=high grain yield to 5=low grain yield), ER: Ear Rot (1=no ears rotted to 5=ears mostly rotted), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), HT: Husk Tightness (1=tight to 5=loose), HC: Husk Cover (1=long to 5=short), PA: Plant aspect (1=desirable to 5=undesirable), PH: distance from ground to the top of the tassel, EH: distance from ground to point of attachment of ear shank.

<sup>¶</sup>Mean separations determined using the logarithmic transformation of the data

<sup>§</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

Table 3-5. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at Weslaco, TX in 2000.

Entry	Pedigree	Rank <sup>†</sup>	AF	MF	FF	ASI	GY	EA	TXT	ER	HT	HC	SLOD	PH	EH
			ng g <sup>-1</sup>	d	d	d	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	%	in	in
1	CML 326/Mp420	10	190.5 c-e	67.0	71.5	4.5	3.5	3.6	1.9	3.1	3.5	2.9	0.0	88.0	30.0
2	TX772/Mp420	5	120.2 c-e	67.0	69.3	2.3	3.3	3.3	2.1	2.8	2.9	1.6	0.6	89.5	30.0
3	CML285/Mp420	9	182.0 c-e	70.0	72.0	2.0	2.1	3.1	4.0	2.5	3.8	2.6	5.7	97.2	40.0
4	Tx601y/Mp420	14	309.0 b-d	70.5	76.0	5.5	2.4	3.6	3.9	2.8	2.1	1.4	3.8	103.4	41.0
5	FR2128/Mp420	17	331.1 b-d	68.5	71.5	3.0	2.8	2.4	3.1	2.8	2.9	1.9	0.0	89.5	39.0
6	TX772/CML326	2	58.9 e	67.0	69.3	2.3	2.9	2.5	1.1	2.6	3.3	2.0	0.0	77.5	28.0
7	CML285/CML326	6	120.2 c-e	70.0	74.0	4.0	2.3	2.3	2.5	2.0	3.3	3.1	0.0	93.1	33.0
8	Tx601y/CML326	15	309.0 b-d	70.0	76.0	6.0	1.8	1.6	2.3	1.5	2.6	2.4	0.6	96.6	35.0
9	FR2128/CML326	18	407.4 b,c	69.3	71.5	2.3	2.8	1.6	2.0	1.8	2.5	1.8	0.0	86.6	27.0
10	CML285/TX772	7	131.8 c-e	69.3	73.5	4.3	1.5	2.6	2.3	1.5	2.1	1.5	0.0	90.7	38.0
11	Tx601y/TX772	8	166.0 c-e	70.0	75.0	5.0	1.8	3.1	2.0	2.3	3.4	3.0	0.6	90.7	36.0
12	FR2128/TX772	11	213.8 c-e	67.0	70.0	3.0	2.8	1.8	1.0	3.4	3.6	2.4	0.0	82.4	32.0
13	Tx601y/CML285	4	109.6 c-e	75.0	76.0	1.0	1.8	3.8	4.1	2.1	1.6	1.6	3.8	102.1	45.0
14	FR2128/CML285	13	281.8 b-d	70.0	72.0	2.0	2.1	1.6	3.5	1.9	3.6	2.9	1.4	90.6	33.0
15	FR2128/Tx601y	19	955.0 a,b	70.0	75.0	5.0	2.4	2.8	3.4	2.0	2.8	2.1	0.6	96.1	42.0
16	Tx601y/Mp715	3	93.3 d,e	76.0	80.0	4.0	4.8	5.0	3.9	2.8	1.9	1.5	4.1	100.9	53.0
17	FR2128/Mp715	12	234.4 c-e	74.0	76.0	2.0	3.8	4.1	2.9	2.9	3.1	3.5	0.6	92.9	48.0
18	TX772/Mp715	1	10.7 f	71.0	76.0	5.0	2.9	2.8	1.9	2.1	2.4	1.5	0.6	89.8	44.0
19	DK668	16	309.0 b-d	67.8	70.3	2.5	2.0	1.5	4.0	2.0	3.3	2.0	0.0	81.0	33.0
20	P3223	20	1819.7 a	70.0	71.0	1.0	2.4	2.5	4.0	3.9	4.6	4.0	0.0	83.7	32.0
	Mean		317.7	70.0	73.3	3.3	2.6	2.8	2.8	2.4	3.0	2.3	1.1	91.1	37.0
	LSD <sup>§</sup>			1.7	2.3	2.6	1.2	1.1	0.5	1.3	1.0	0.8	2.9	3.8	0.0
	Significance		**	***	***	**	***	***	***	***	***	***	***		
	C.V. % <sup>#</sup>		18.7	1.5	2.0	47.6	27.6	24.9	10.1	31.6	20.4	21.3	159.8	2.6	0.0

\* , \*\* and \*\*\* Significant at .05, .01 and .001 Levels, respectively, NS=Not Significant at .05 level

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, GY: visual rating for grain yield (1=high grain yield to 5=low grain yield), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), HT: Husk Tightness (1=tight to 5=loose), HC: Husk Cover (1=long to 5=short), LD: Lodging (1=all plants standing erect to 5=all plants lodged), PA: Plant Aspect (1=desirable to 5=undesirable), AFR: visual rating for *Aspergillus flavus* Colonization (1= no colonization to 5=all ears colonized)

<sup>§</sup>Fisher's least significant difference

<sup>¶</sup>Mean separations determined using the logarithmic transformation of the data

<sup>#</sup>C.V. %: Coefficient of Variation



*Corpus Christi 2000*

The mean aflatoxin accumulation for yellow hybrids at Corpus Christi, TX was 424.3 ng g<sup>-1</sup> with a range of 100.2 ng g<sup>-1</sup> to 2194.3 ng g<sup>-1</sup> (Table 3-6). Significant differences were detected for all traits measured. Two commercial hybrids (DKXL269 and P30R39) were part of three hybrids less susceptible for aflatoxin accumulation. Both of these hybrids are of tropical origins and have good husk cover and flintier kernels (Gary Odvody, personal communications).

Hybrid DKXL269 had the lowest insect damage rating (1.5), a lower than average grain yield rating (2.3), a low grain texture rating (1.8) and a higher than average ear weight (70.1 g ear<sup>-1</sup>) (Table 3-6). Hybrid FR2128/Mp715 had a lower than average insect damage rating (2.7), higher than average grain yield rating (2.9), lower than average grain texture rating (2.6) and an average ear weight (65.4 g ear<sup>-1</sup>) (Table 3-6). Hybrid P30R39 had the second lowest ear aspect rating (1.8), lower than average insect damage rating (2.1), the second lowest grain yield rating (1.6), higher than average grain texture rating (3.5) and higher than average ear weight (70.8 g ear<sup>-1</sup>) (Table 3-6).

Hybrids composed of lines previously shown to reduce aflatoxin were accumulating rather large amounts of the toxin. Traits other than the ones measured must also affect accumulation of aflatoxin in maize.

Table 3-6. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at Corpus Christi, TX in 2000.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	LD 1 to 5	EA 1 to 5	ID 1 to 5	GY 1 to 5	TXT 1 to 5	EW g ear <sup>-1</sup>
1	CML 326/Mp420	21	675.6 b-e <sup>§</sup>	1.8	2.7	2.9	2.6	1.8	65.8
2	TX772/Mp420	18	653.1 b-e	4.6	3.5	3.6	3.2	1.8	66.2
3	CML285/Mp420	12	399.3 e-h	3.4	2.9	2.3	2.6	4.5	70.8
4	Tx601y/Mp420	20	666.8 b-e	3.3	4.2	4.1	4.0	4.2	49.9
5	FR2128(B73)/Mp420	11	383.7 e-h	1.7	3.7	3.4	3.6	2.8	55.5
6	TX772/CML326	8	255.3 f-j	1.7	2.4	2.6	2.2	1.3	65.7
7	CML285/CML326	9	292.2 f-j	1.8	1.6	1.6	2.0	1.9	68.4
8	Tx601y/CML326	7	253.5 g-j	1.7	2.4	2.3	2.4	2.1	61.8
9	FR2128(B73)/CML326	4	199.5 i-k	1.2	1.6	1.8	1.5	1.6	82.1
10	Tx714/TX772	22	830.4 b-d	2.4	3.0	3.4	2.6	2.2	65.7
11	Tx601y/TX772	17	631.0 b-e	3.7	3.1	3.1	3.0	1.8	60.8
12	FR2128(B73)/TX772	14	476.1 d-f	1.3	2.0	2.2	2.1	1.6	71.1
13	Tx601y/CML285	13	457.1 d-g	2.6	3.6	2.5	3.4	4.5	50.6
14	FR2128(B73)/CML285	10	364.2 e-i	1.4	2.0	1.8	2.1	3.6	75.8
15	FR2128(B73)/MAS gk der.line	19	663.7 b-e	1.7	3.3	3.2	3.2	2.5	68.2
16	Tx601y/Mp715	5	220.8 h-k	3.1	4.7	3.5	4.6	4.5	29.9
17	FR2128(B73)/Mp715	2	118.7 k,l	3.0	3.2	2.7	2.9	2.6	65.4
18	MAS gk/TX772	24	1126.4 b	2.3	3.7	4.1	3.1	1.9	60.4
19	MP420/Mp715	6	223.7 h-j	3.8	3.6	3.5	3.1	4.4	57.9
20	MAS-gk/CML326	15	592.9 c-e	1.4	2.3	3.0	2.4	1.9	67.5
21	MAS gk/Mp420	25	2194.3 a	3.1	4.8	4.6	4.7	4.3	42.2
22	C7997	16	619.9 b-e	1.7	4.1	4.7	3.3	4.8	56.4
23	P30R39	3	193.8 j,k	1.8	1.8	2.1	1.6	3.5	70.8
24	DKXL269	1	100.2 l	2.2	2.0	1.5	2.3	1.8	70.1
25	P31B13BT	23	1015.5 b,c	1.5	3.1	3.8	1.2	3.6	100.4

Table3-6. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	LD	EA	ID	GY	TXT	EW
			ng g <sup>-1</sup>	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	g ear <sup>-1</sup>
	Mean		424.3	2.3	3.0	3.0	2.8	2.9	64.0
	LSD <sup>¶</sup>			0.9	0.5	0.6	0.5	0.3	8.7
	Sig.		***	***	***	***	***	***	***
	C.V.% <sup>#</sup>		5.5	43.2	19.3	22.3	19.6	12.5	14.6

\*\*\* Significant at .001 level

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, LD: visual rating for lodged plants (1=all plants standing erect to 5=most plants either lodged below the ear node or leaning past 30° past vertical), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), ID: visual rating for insect damage to kernels on ear (1=few ears damaged to 5=most ears damaged with channeling ), GY: visual rating for grain yield (1=high grain yield to 5=low grain yield), TXT: Grain Texture (1=flint to 5=dent), EW: grams per ear.

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

### Across Locations 2000

Seventeen hybrids and two locations were included in the across location analysis for aflatoxin and secondary traits in 2000. The mean aflatoxin across locations was  $135.1 \text{ ng g}^{-1}$  with a range of  $40.5 \text{ ng g}^{-1}$  to  $244.8 \text{ ng g}^{-1}$  (Table 3-7). Differences were not detected for aflatoxin and silking date. Significant differences were detected for husk cover. Hybrid Tx772/Mp715 had the lowest numerical accumulation of aflatoxin ( $40.5 \text{ ng g}^{-1}$ ), had a low husk cover (1.8) rating and a longer than average silking date (74.5 d). Hybrid Tx772/CML326 had the second lowest numerical accumulation of aflatoxin ( $60.9 \text{ ng g}^{-1}$ ), the second lowest husk cover (1.4) rating and the lowest silking date (69.5 d).

Significant genotype\*environment interaction were detected for aflatoxin, husk cover and silking date for yellow hybrids across three locations in 2000 (Figure 3-2).

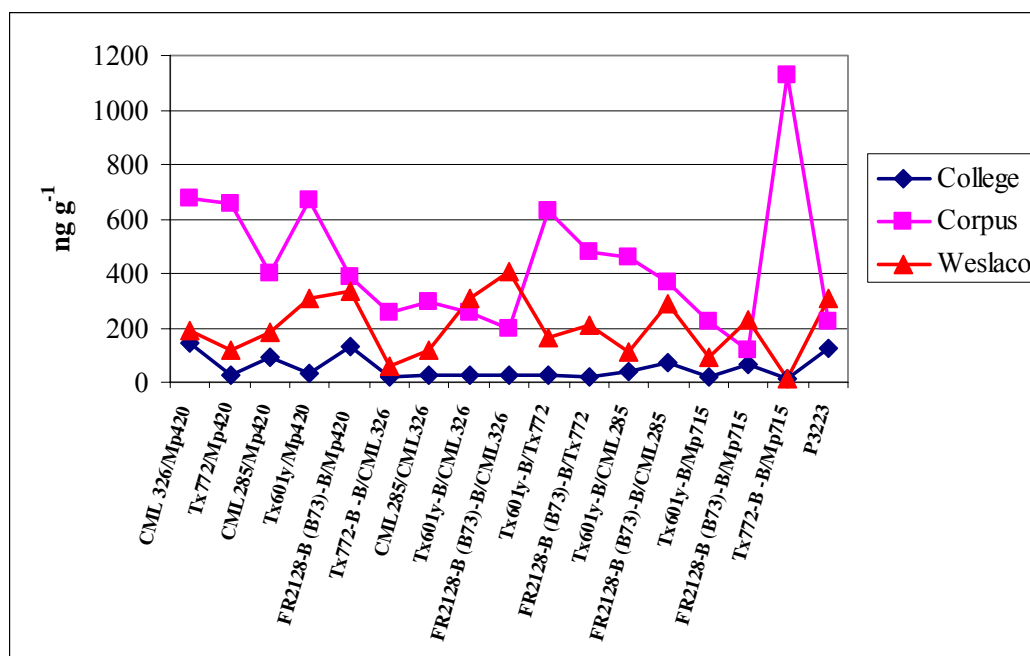


Figure 3-2. Aflatoxin accumulation in yellow hybrids across locations in 2000.

Table 3-7. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids across three locations in 2000.

Entry	Pedigree	Rank <sup>‡</sup>	AF <sup>†</sup>		HC		FF	
			Mean	BLUP <sup>¶</sup>	Means	BLUP	Means	BLUP
			ng g <sup>-1</sup>	ng g <sup>-1</sup>	1 to 5	1 to 5	d	d
1	CML 326/Mp420	16	244.8	137.5	1.7	1.9	70.7	72.8
2	TX772/Mp420	5	107.3	119.8	1.6	1.9	71.3	72.9
3	CML285/Mp420	13	176.8	129.8	3.1	2.8	72.4	73.0
4	Tx601y/Mp420	12	163.9	128.6	2.9	2.7	76.8	73.3
5	FR2128/Mp420	17	246.0	137.1	2.1	2.2	71.9	72.9
6	TX772/CML326	2	60.9	108.3	1.4	1.8	69.5	72.7
7	CML285/CML326	4	87.6	115.2	2.1	2.2	73.0	73.0
8	Tx601y/CML326	11	121.2	121.5	2.0	2.1	75.2	73.2
9	FR2128/CML326	8	118.3	120.8	2.0	2.1	71.7	72.9
11	Tx601y/TX772	9	120.8	122.2	1.9	2.0	74.4	73.1
12	FR2128/TX772	7	114.6	120.9	1.2	1.7	70.4	72.8
13	Tx601y/CML285	6	111.9	120.4	3.1	2.8	76.8	73.3
14	FR2128/CML285	14	185.6	130.7	2.8	2.6	72.1	72.9
16	Tx601y/Mp715	3	70.3	110.9	3.5	3.0	73.1	73.1
17	FR2128/Mp715	10	121.1	120.9	2.9	2.6	74.7	73.1
18	TX772/Mp715	1	40.5	102.3	1.8	2.0	74.5	73.1
20	P3223	15	204.4	132.4	3.1	2.8	72.5	73.0
Mean			135.1	122.3	2.4	2.3	73.6	73.0
LSD					0.3		1.9	
Genotype Sig.			NS		*		NS	
Environment Sig.			***		***		***	
G*E Sig.			**		***		***	
C.V. % <sup>#</sup>			20.8		16.5		3.1	

\*\* and \*\*\* Significant at .01 and .001 Levels, respectively

NS=Not significant at .05 level

<sup>†</sup>Traits are: AF=antilogarithmic transformation of data, FF=days from planting to silking, HC=Husk

<sup>‡</sup>Rank of genotypes by Aflatoxin concentration

Cover (1=good husk cover and 5=poor husk cover)

<sup>¶</sup>BLUP=Best Linear Unbiased Predictor

<sup>§</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of variation

*College Station 2001*

The mean aflatoxin accumulation for yellow hybrids at College Station in 2001 was  $89.6 \text{ ng g}^{-1}$  with a range of  $9.4 \text{ ng g}^{-1}$  to  $359.1 \text{ ng g}^{-1}$  (Table 3-8). Significant differences were detected for all traits measured. Subtropical lines are parents in two of the three least susceptible hybrids. Line Tx601y crossed with line NC300 was the least susceptible hybrid. Temperate line Tx770 crossed with subtropical line CML325 was the second least susceptible hybrid for aflatoxin accumulation. Temperate line Tx772 crossed with subtropical line CML323 was the third least susceptible hybrid for aflatoxin accumulation.

Hybrid Tx601Y/NC300 had a later than average silking date (81 d), a tie for the lowest ear aspect rating (1.38), the best visual rating for grain yield (1.38) and the third lowest husk cover rating (1.16). Hybrid Tx770/CML325 had a lower than average silking date (77.75 d), attained similar ear aspect rating (3.00) to four other hybrids, an average grain texture rating (2.13), and a relatively poor husk cover (3.08). Hybrid Tx772/CML323 had an one of the earliest flowing dates (76.0 d), a better than average ear aspect rating (2.25), a above average visual rating for grain yield (2.75), the best rating for grain texture (1.0) and a poor rating for husk cover (3.18).

Tx772 was in five hybrid combinations and each hybrid combination was within the lowest 24 ranked hybrids (range of  $14.5 \text{ ng g}^{-1}$  to  $56.7 \text{ ng g}^{-1}$ ), all statistically similar to the least susceptible hybrid. This group of Tx772 hybrids, Tx601y, a parent in the least susceptible hybrid in this trial, was also a parent in the most susceptible combination as well.

Table 3-8. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at College Station, TX in 2001

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	GY	EA	TXT	HC
			ng g <sup>-1</sup>	d	1 to 5	1 to 5	1 to 5	1 to 5
1	Tx732/Tx770	11	31.7 c-i <sup>§</sup>	79.00	1.88	2.00	2.63	2.52
2	Tx732/NC300	19	43.7 b-i	77.00	2.88	2.75	2.38	1.82
3	(LH252xLH262)/Tx732	16	38.7 b-i	76.25	1.88	2.50	3.63	2.92
4	Tx732/CML285	38	230.3 a,b	82.00	2.13	1.75	2.63	2.66
5	Tx732/CML288	14	37.2 b-i	81.25	2.13	2.38	1.88	3.18
6	Tx732/CML289	29	132.8 a-f	78.25	3.63	3.63	2.00	2.08
7	Tx732/CML294	36	203.7 a-c	82.50	2.38	2.63	1.88	3.95
8	Tx732/CML323	5	21.0 f-i	76.00	2.88	2.63	1.88	3.68
9	Tx732/CML325	22	50.1 a-i	76.25	2.88	3.38	3.38	2.38
10	Tx732/CML338	6	22.79 f-i	77.75	3.00	3.00	2.13	1.36
11	CML323/Tx770	10	29.7 c-i	78.25	2.50	2.50	1.63	3.10
12	NC300/Tx770	33	150.7 a-f	82.25	3.00	2.50	2.50	1.65
13	FR2128/Tx770	37	223.9 a,b	80.25	2.25	2.13	2.00	1.73
14	Tx770/CML285	12	34.4 b-i	82.50	1.88	2.13	2.38	2.69
15	Tx770/CML288	32	150.3 a-f	83.25	1.63	2.13	2.00	3.85
16	Tx770/CML289	30	134.1 a-f	83.25	2.63	2.50	2.00	3.59
17	Tx770/CML294	26	87.7 a-h	83.00	2.50	2.75	2.00	3.35
18	Tx770/CML325	2	12.4 i	77.75	3.00	2.75	2.13	3.08
19	CML338/TX770	18	41.4 b-i	81.00	2.25	2.75	1.88	1.58
20	Tx714/TX772	7	23.3 e-i	76.50	2.13	2.00	1.88	1.19
21	(LH235xLH236)/TX772	24	56.7 a-i	77.50	2.25	1.88	1.63	1.71
22	CML 161 X CML 170	4	16.9 g-i	81.75	2.75	3.13	1.00	3.14
23	TX772/CML323	3	14.5 h,i	76.00	2.25	2.75	1.00	3.18
24	FR2128/TX772	21	47.9 b-i	77.50	3.75	3.75	1.13	0.97
25	Tx601y/TX772	13	36.8 b-i	82.50	1.63	1.88	1.25	1.44
26	FR2128/CML323	17	38.8 b-i	78.25	2.13	2.38	1.38	1.73
27	NC300/FR2128	20	46.7 a-i	81.50	2.00	2.38	2.13	1.12
28	FR2128/CML285	9	27.3 d-i	82.00	2.00	2.00	1.88	2.52

Table 3-8. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	GY	EA	TXT	HC
			ng g <sup>-1</sup>	d	1 to 5	1 to 5	1 to 5	1 to 5
29	FR2128/CML289	8	24.9 d-i	80.00	3.13	3.63	2.00	1.62
30	FR2128/CML294	28	119.2 a-g	81.50	2.88	2.00	1.50	3.27
31	FR2128/CML338	25	60.6 a-i	80.75	2.63	2.50	1.63	1.32
32	FR2128/Tx770	35	170.9 a-d	82.00	1.88	2.38	2.00	1.82
33	Tx601Y/NC300	1	9.4 i	81.00	1.38	1.38	2.63	1.16
34	Tx601Y/CML323	23	53.9 a-i	80.50	1.63	1.38	1.50	2.53
35	Tx601y/CML285	40	359.1 a	86.00	1.88	1.75	3.13	1.74
36	[CML 161 X G26Qc18MH134-4- #-3-#-#-B-B-B] X DO 940Y	31	141.3 a-f	83.75	3.25	3.25	1.75	2.69
37	Pioneer 3223	39	246.6 a,b	78.50	2.38	3.88	2.75	3.08
38	DK668	15	38.7 b-i	77.00	3.13	3.00	4.00	1.59
39	RX889	34	165.7 a-e	76.75	2.75	2.63	3.75	2.05
40	8285 7Y35	27	99.4 a-h	77.25	3.38	3.13	4.00	1.33
	Mean		86.9	79.9	2.5	2.5	2.2	2.3
	LSD <sup>¶</sup>		.	2.1	1.0	0.9	0.4	0.7
	Significance		**	***	***	***	***	***
	C.V. % <sup>#</sup>		34.2	1.9	28.5	27.1	13.0	20.8

\*, \*\*, \*\*\* Significant at the .05, .01 and .001 levels, respectively

<sup>†</sup>Rank of genotypes by aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), GY: visual rating for grain yield (1=high grain yield to 5=low grain yield, TXT: Grain Texture (1=flint to 5=dent), HC: Husk Cover (1=long to 5=short)

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation



### *Weslaco 2001*

The mean aflatoxin accumulation for yellow hybrids at Weslaco in 2001 was 361.3 ng g<sup>-1</sup> with a range of 75.08 ng g<sup>-1</sup> to 1796.80 ng g<sup>-1</sup> (Table 3-9). Significant differences were detected for all traits measured. The three hybrids (Tx601y/NC300, CML161/CML170, and Tx770/CML325) less susceptible for aflatoxin accumulation were comprised of subtropical or tropical origins.

Hybrid Tx601y/NC300 had a wide anthesis silking interval (3.0 d), tied for the second lowest ear aspect rating (1.5), an average grain texture rating (2.63), a good husk cover rating (1.38), second lowest insect damage rating (1.75), and a low visual *Aspergillus flavus* colonization rating (1.88). Hybrid CML161/CML170 had an above average anthesis silking interval (2.25 d), a high ear aspect rating (3.13), the second lowest grain texture rating (1.50), a relatively poor husk cover rating (3.63), one of the third lowest insect damage ratings (1.88) and a low visual *A. flavus* colonization rating (1.63). Hybrid Tx770/CML325 had a relatively long anthesis silking interval (3.75 d), above average ear aspect rating (2.5), grain texture rating (2.88), husk cover rating (4.38), insect damage rating (3.0) and a below average visual *A. flavus* colonization rating (2.13).

Table 3-9. Means and statistics for aflatoxin concentration and secondary traits of yellow hybrids at Weslaco, TX in 2001.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	MF days	FF days	ASI days	GY 1 to 5	EA 1 to 5	TXT 1 to 5	HC 1 to 5	ID 1 to 5	AFR 1 to 5
1	Tx732/Tx770	4	192.2g-k <sup>§</sup>	65.50	67.00	1.50	2.50	2.38	4.00	3.38	2.63	2.25
2	Tx732/NC300	8	247.9f-j	64.00	67.00	3.00	2.13	2.13	3.25	2.63	2.38	2.13
3	(LH252xLH262)/Tx732	21	309.9e-j	64.00	66.25	2.25	2.50	2.50	3.63	3.50	3.00	3.13
4	Tx732/CML285	40	1796.8a	69.25	68.50	0.00	2.75	2.75	3.88	3.00	2.75	2.63
5	Tx732/CML288	23	443.4c-i	66.25	67.00	0.75	2.50	2.88	2.50	3.75	3.00	2.50
6	Tx732/CML289	27	465.3c-i	67.00	67.75	0.75	2.13	2.38	2.13	3.38	2.38	2.00
7	Tx732/CML294	32	530.6b-g	69.25	70.00	0.75	1.88	1.88	2.13	4.50	2.38	2.38
8	Tx732/CML323	6	175.4i-k	62.50	65.50	3.00	2.63	2.25	1.75	3.75	2.88	2.25
9	Tx732/CML325	31	579.1b-f	61.00	64.00	3.00	3.25	3.00	3.00	3.13	3.00	2.50
10	Tx732/CML338	7	177.4h-k	64.00	66.25	2.25	2.63	2.75	2.63	2.00	2.00	2.13
11	CML323/Tx770	34	597.7b-f	65.50	67.75	2.25	2.75	3.13	2.00	4.00	2.00	2.50
12	NC300/Tx770	26	302.9e-j	66.25	70.00	3.75	1.75	2.13	3.00	3.13	2.50	2.25
13	FR2128/Tx770	24	401.1c-i	69.25	70.75	1.50	2.63	2.75	2.50	2.13	2.63	2.75
14	Tx770/CML285	30	386.4c-i	70.00	67.75	0.00	2.00	2.13	4.38	3.50	2.38	2.00
15	Tx770/CML288	28	473.4c-i	70.00	71.50	1.50	1.88	1.88	2.38	3.13	2.00	1.75
16	Tx770/CML289	33	616.2b-f	70.75	70.75	0.00	2.00	2.38	2.50	5.00	2.63	2.63
17	Tx770/CML294	15	276.9f-j	70.00	70.75	0.75	2.75	2.50	3.25	4.13	2.13	2.00
18	Tx770/CML325	3	175.3i-k	64.00	67.75	3.75	2.25	2.50	2.88	4.38	3.00	2.13
19	CML338/TX770	5	176.8h-k	67.00	69.25	2.25	2.75	2.50	2.25	2.63	2.50	2.13
20	Tx714/TX772	38	810.0a-e	64.00	67.75	3.75	2.13	2.00	2.00	1.25	2.00	1.88
21	(LH235xLH236)/TX772	11	301.8e-j	64.75	68.50	3.75	1.88	2.13	1.75	1.38	2.75	2.00
22	CML161/CML170	2	132.2j,k	67.00	69.25	2.25	3.50	3.13	1.50	3.63	1.88	1.63
23	TX772/CML323	39	1407.7a,b	61.75	64.00	2.25	2.13	1.50	1.00	3.25	1.63	1.38
24	FR2128/TX772	19	327.7d-j	64.00	68.50	4.50	2.63	2.63	1.38	1.38	2.75	2.75
25	Tx601y/TX772	9	221.6f-j	67.75	70.75	3.00	1.75	1.75	2.25	2.50	2.50	1.75
26	FR2128/CML323	36	952.8a-c	64.00	67.75	3.75	3.75	3.50	1.63	2.00	2.25	3.38
27	NC300/FR2128	29	418.3 b-g <sup>§</sup>	65.50	69.25	3.75	1.38	1.50	2.13	2.25	1.88	1.63

Table 3-9. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	MF days	FF days	ASI days	GY 1 to 5	EA 1 to 5	TXT 1 to 5	HC 1 to 5	ID 1 to 5	AFR 1 to 5
28	FR2128/CML285	20	361.8 c-i	70.00	68.50	0.00	2.25	2.50	2.38	3.13	1.75	1.88
29	FR2128/CML289	22	288.1 c-j	70.00	70.00	0.00	2.00	1.88	2.13	2.75	2.00	2.50
30	FR2128/CML294	17	292.6 f-j	70.00	70.75	0.75	3.50	3.00	1.38	3.88	1.25	1.25
31	FR2128/CML338	13	946.2 e-j	65.50	68.50	3.00	2.88	2.75	1.75	1.25	2.50	2.00
32	FR2128/Tx770	35	75.1 a-c	69.25	71.50	2.25	2.13	2.13	2.38	3.38	2.63	2.88
33	Tx601Y/NC300	1	226.3 k	67.75	70.75	3.00	1.75	1.50	2.63	1.38	1.75	1.88
34	Tx601Y/CML323	16	192.2 f-j	66.25	70.00	3.75	1.25	1.25	1.63	2.88	1.25	1.25
35	Tx601y/CML285	25	288.4 g-k	71.50	73.25	1.75	1.50	1.50	3.75	1.38	1.88	2.25
36	[CML 161 X G26Qc18MH134-4-#-3-#-#- #-B-B-B] X DO 940Y	14	877.0 f-j	70.00	71.50	1.50	2.63	2.88	1.88	3.38	3.38	3.25
37	Pioneer 3223	37	307.1 a-d	69.25	70.00	0.75	2.25	3.63	3.88	4.50	3.88	3.63
38	DK668	10	292.6 e-j	67.75	68.50	0.75	2.13	2.13	3.88	1.75	1.88	1.75
39	RX889	12	404.3 e-j	64.00	67.75	3.75	2.63	2.38	4.00	3.13	2.00	2.38
40	8285 7Y35	18	448.5 c-i	67.00	67.75	0.75	2.75	2.88	4.00	1.75	2.38	2.00
	Mean		418.3	66.8	68.8	1.9	2.4	2.4	2.6	2.9	2.4	2.2
	LSD <sup>¶</sup>		.	1.7	2.5	2.9	1.1	1.1	0.7	1.0	0.9	1.0
	Sig.		***	***	***	***	**	**	***	***	***	***
	C.V. % <sup>#</sup>		12.5	1.8	2.6	105.2	33.0	18.7	18.7	23.2	27.3	31.8

\*, \*\*, \*\*\* Significant at .05, .01, .001 levels, respectively

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, MF: days from planting to anthesis, FF: days from planting to silking, ASI: anthesis silking interval, GY: visual rating for grain yield (1=high grain yield and 5=low grain yield), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), HC: Husk Cover (1=long to 5=short), ID: insect damage (1=no damage to 5=all ears damage), AFR: visual rating for *Aspergillus flavus* Colonization (1= no colonization to 5=all ears colonized)

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

*Corpus Christi 2001*

The mean aflatoxin accumulation for yellow hybrids at Corpus Christi in 2001 was  $1600.1 \text{ ng g}^{-1}$  with a range of  $76.6 \text{ ng g}^{-1}$  to  $5474.3 \text{ ng g}^{-1}$  (Table 3-10). Significant differences were detected for all traits measured. Two commercial hybrids (P30R39 and DKLX269) used as resistant checks were part of three hybrids less susceptible to aflatoxin accumulation. Tx601y/CML285 was the third least susceptible hybrid.

Pioneer brand Hybrid P30R39 had a longer than average silking date (80.22 d), a high ear aspect rating (4.11), lower than average grain texture rating (2.17), insect damage rating (2.72) and *A. flavus* visual rating (2.22). Dekalb hybrid DKXL269 had a longer than average silking date (78.56 d), below average ear aspect rating (3.06), a relatively low grain texture rating (1.44), below average insect damage rating (2.56) and *A. flavus* visual rating (2.22). Hybrid Tx601Y/CM285 had a longer than average silking date (80.44 d), a high ear aspect rating (4.44) and grain texture rating (3.39), a lower than average insect damage rating (3.11) and *A. flavus* visual rating (2.61). The three hybrids accumulating the least aflatoxin, did not express those traits previously shown to reducing aflatoxin accumulation. However; these three hybrids did accumulate less aflatoxin than other hybrids such as FR2128/CML323 ( $781.0 \text{ ng g}^{-1}$ ).

Table 3-10. Means and statistics of aflatoxin concentrations and secondary traits of yellow hybrids at Corpus Christi, TX in 2001.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	GY	EA	TXT	ID	AFR
			ng g-l	d	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5
1	Tx732/Tx770	30	5474.3 a <sup>§</sup>	76.78	3.67	4.00	4.00	4.28	3.83
2	Tx732/CML285	20	1257.0 e-g	80.78	4.39	4.22	2.94	3.28	2.89
3	Tx732/CML289	9	695.0 g-i	79.00	3.78	2.94	1.94	3.22	2.83
4	Tx732/CML294	8	694.5 g-i	80.11	3.78	3.17	1.67	3.39	2.89
5	Tx732/CML323	17	1126.3 e-h	71.78	2.06	2.72	1.94	3.44	2.67
6	Tx732/CML325	23	2294.4 b-e	72.00	2.50	2.61	3.94	3.67	3.17
7	Tx732/CML338	13	926.1 g-i	78.56	3.78	3.22	1.89	2.94	2.28
8	CML323/Tx770	29	4749.7 a,b	72.56	2.61	3.11	2.00	3.94	3.83
9	NC300/Tx770	7	462.7 i-k	80.56	4.06	4.17	4.06	3.33	2.89
10	Tx770/CML285	19	1263.8 e-g	80.33	4.39	3.72	3.72	3.00	2.44
11	Tx770/CML289	14	942.6 f-i	80.33	4.06	3.11	3.00	2.94	2.33
12	Tx770/CML294	15	955.7 f-i	80.33	4.28	3.89	2.17	3.78	2.89
13	Tx770/CML325	21	2049.6 c-f	73.00	3.06	3.00	2.83	3.61	3.06
14	CML338/TX770	28	3929.4 a-c	72.67	3.00	3.33	2.33	4.00	3.50
15	Tx714/TX772	18	1350.0 d-g	72.78	3.89	3.94	1.72	4.44	4.39
16	TX772/CML323	22	2192.8 b-e	70.00	1.94	2.89	1.22	4.44	3.89
17	FR2128/TX772	16	982.5 f-i	71.44	2.33	2.39	1.17	3.61	2.89
18	Tx601y/TX772	25	2807.6 a-d	80.56	4.50	4.50	1.33	4.17	3.78
19	FR2128/CML323	11	781.0 g-i	71.56	1.89	1.89	1.50	2.67	2.11
20	NC300/FR2128	4	476.8 i-k	76.67	2.39	2.72	2.06	2.00	1.83
21	FR2128/CML285	10	553.8 h-k	79.56	3.83	3.50	1.83	2.67	2.00
22	FR2128/CML289	6	634.8 g-j	76.67	4.06	3.94	1.89	3.44	3.17
23	FR2128/CML294	5	547.9 h-k	78.89	2.78	3.17	1.33	3.44	2.94
24	FR2128/CML338	12	794.3 g-i	71.22	2.17	2.33	3.11	2.22	2.17
25	FR2128/Tx770	24	2257.7 b-e	77.78	3.61	3.33	2.50	3.72	3.22
26	Tx601y/CML285	3	302.5 j,k	80.44	4.72	4.44	3.39	3.11	2.61
27	P3223	26	3152.6 a-c	69.44	2.67	4.11	2.94	4.56	4.33
28	P30R39	1	76.6 l	80.22	4.39	4.11	2.17	2.72	2.22

Table 3-10. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	GY	EA	TXT	ID	AFR
			ng g <sup>-1</sup>	d	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5
29	DKXL269	2	282.5 k	78.56	3.44	3.06	1.44	2.56	2.22
30	P31B13BT	27	3987.2 a-c	69.11	1.22	3.33	3.22	4.28	4.00
	Mean		1600.1	76.1	3.3	3.4	2.4	3.4	3.0
	LSD <sup>¶</sup>		.	3.32	0.73	0.81	0.83	0.66	0.78
	Sig.		***	***	***	***	***	***	***
	C.V. % <sup>#</sup>		6.8	4.70	23.71	25.99	37.64	20.75	28.06

\*\*\* Significant at .001 levels, respectively

<sup>†</sup>Rank of genotypes by aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, GY: visual rating for grain yield (1=high grain yield to 5=low grain yield), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), ID: Insect damage (1=no damage or channeling to 5=heavy damage or channeling), AFR: visual rating for *Aspergillus flavus* Colonization (1= no colonization to 5=all ears colonized)

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

*Across Locations 2001*

Twenty seven yellow hybrids and three locations were included in the across location analysis in 2001. The mean aflatoxin accumulation across environments was  $304.7 \text{ ng g}^{-1}$  with a range of  $131.3 \text{ ng g}^{-1}$  to  $779.1 \text{ ng g}^{-1}$  (Table 3-11). No significant differences were detected for aflatoxin across environments. Significant differences were, however; detected for grain texture and silking date.

Line Tx770 crossed with subtropical line CML325 had lower than average aflatoxin accumulation ( $131.3 \text{ ng g}^{-1}$ ), higher than average grain texture rating (2.5) and lower than average silking date (73.7 d) (Table 3-11). Line Tx732 crossed with tropical line CML338 had lower than average aflatoxin accumulation ( $131.4 \text{ ng g}^{-1}$ ), lower than average grain texture rating (1.8) and lower than average silking date (72.3 d) (Table 3-11). Line Tx732 crossed with subtropical line CML323 had lower than average aflatoxin ( $136.1 \text{ ng g}^{-1}$ ), lower than average grain texture rating (2.2) and higher than average silking date (72.3 d) (Table 3-11). Hybrid Tx770/CML325 accumulated much less aflatoxin at College Station and Weslaco than in Corpus Christi.

Significant differences were detected for genotype\*environment interaction. The AMMI biplot for aflatoxin accumulation shows the stability of hybrids in reference to the locations and how the locations are related (Figure 3-3).

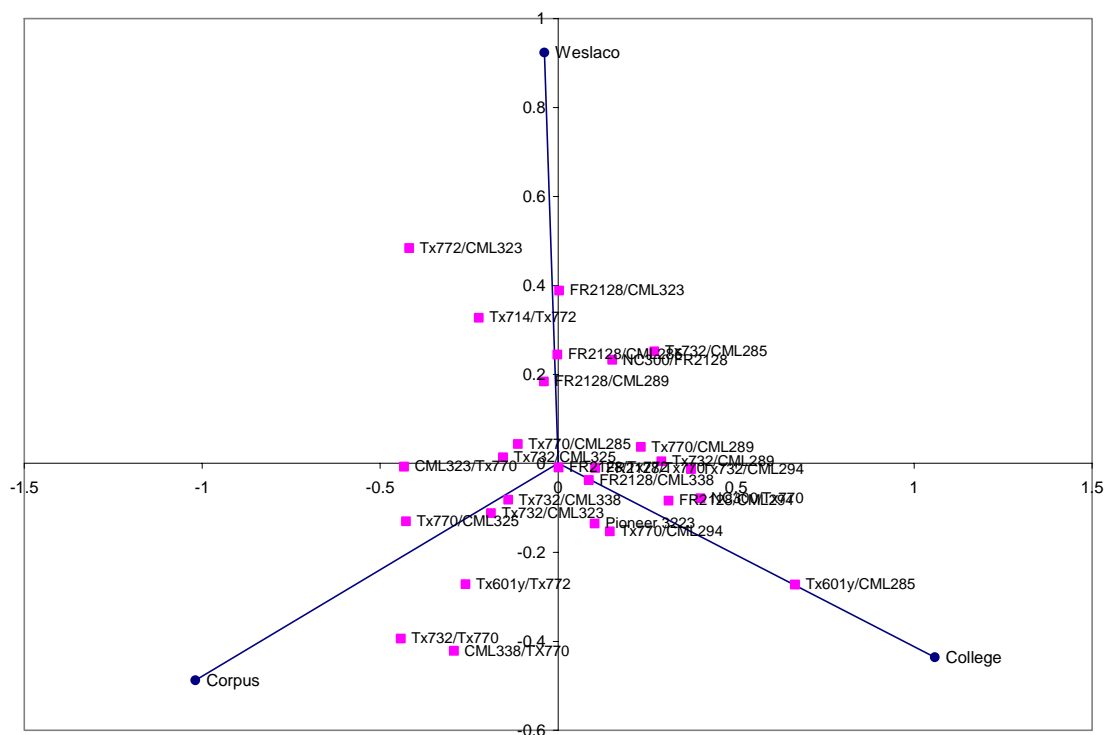


Figure 3-3. AMMI biplot for aflatoxin accumulation of yellow hybrids across locations in 2001.

The most stable hybrids for aflatoxin accumulation across environments are near the origin of the graph. The higher the perpendicular projection of a hybrid on the location vector, the more aflatoxin is accumulated by the hybrid at that location. For example, hybrid Tx601y/CML285 accumulated the highest level of aflatoxin at College Station, while at Corpus Christi accumulated similar levels but was placed in lower ranks.



Table 3-11. Means and statistics for aflatoxin concentration and secondary traits of yellow hybrids across three locations in 2001.

Entry	Pedigree	Rank <sup>‡</sup>	AF <sup>†</sup>	TXT		FF	
			Mean	Mean	BLUP <sup>¶</sup>	Mean	BLUP
			ng g <sup>-1</sup>	1 to 5	1 to 5	days	days
1	Tx732/Tx770	11	244.5	3.5	3.4	74.0	74.3
4	Tx732/CML285	26	767.5	3.2	3.1	76.7	76.3
6	Tx732/CML289	20	329.5	2.0	2.1	75.5	75.5
7	Tx732/CML294	23	399.3	1.9	1.9	77.5	76.9
8	Tx732/CML323	3	136.1	1.8	1.9	72.3	73.1
9	Tx732/CML325	22	352.0	3.4	3.3	70.5	71.7
10	Tx732/CML338	2	131.4	2.2	2.2	74.2	74.5
11	CML323/Tx770	21	348.7	1.9	2.0	74.2	74.4
12	NC300/Tx770	16	262.5	3.1	3.1	77.3	76.7
14	Tx770/CML285	9	224.0	3.5	3.4	76.5	76.2
16	Tx770/CML289	24	401.6	2.5	2.5	77.9	77.2
17	Tx770/CML294	15	258.1	2.5	2.5	77.7	77.0
18	Tx770/CML325	1	131.3	2.5	2.5	73.7	74.1
19	CML338/TX770	13	248.7	2.0	2.1	73.8	73.9
20	Tx714/TX772	14	255.7	1.9	1.9	71.6	72.4
23	TX772/CML323	19	305.0	1.0	1.2	70.4	71.5
24	FR2128/TX772	8	218.7	1.2	1.3	72.3	72.8
25	Tx601y/TX772	10	232.3	1.6	1.7	77.7	77.0
26	FR2128/CML323	18	284.4	1.5	1.6	73.0	73.4
27	NC300/FR2128	6	210.2	2.1	2.1	75.0	74.9
28	FR2128/CML285	5	166.9	2.1	2.1	75.8	75.6
29	FR2128/CML289	4	161.3	2.0	2.1	75.4	75.2
30	FR2128/CML294	12	245.7	1.5	1.5	77.2	76.6
31	FR2128/CML338	7	212.2	1.7	1.7	72.9	73.2
32	FR2128/Tx770	25	653.1	2.3	2.3	76.9	76.3
35	Tx601y/CML285	17	267.3	3.4	3.3	79.8	78.5
37	Pioneer 3223	27	779.1	3.1	3.0	72.8	73.2
Mean			304.7	2.3	2.3	74.9	74.9
LSD <sup>§</sup>			.	0.4		2.2	
Gen. Sig.			NS	***		***	
Env. Sig.			***	***		***	
Gen.*Env. Sig			***	***		***	
C.V. % <sup>#</sup>			17.7	18.6		3.4	

\*, \*\*, \*\*\* Significant at .05, .01, .001 levels, respectively

†Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint, 5=dent), FF: days from planting to silking,

‡Rank of genotypes by Aflatoxin concentration

¶Best Linear Unbiased Predictor

§Fisher's least significant difference

#C.V. %: Coefficient of Variation

### *College Station 2002*

The mean aflatoxin accumulation for yellow hybrids at College Station in 2002 was  $4.3 \text{ ng g}^{-1}$  with a range of  $0.0 \text{ ng g}^{-1}$  to  $25.9 \text{ ng g}^{-1}$  (Table 3-12). Significant differences were detected for grain texture, plant appearance and grain yield. No significant differences were detected for aflatoxin accumulation. Aflatoxin accumulation numbers were exceptionally low at College Station in 2002. The ground inoculation with colonized kernels and environmental conditions (i.e., rain and irrigation) could be potential causes of poor colonization and aflatoxin production.

Hybrid Tx714/Tx772 had the second lowest rating for grain texture (1.3), below average plant appearance rating (2.0), and above average grain yield ( $3.2 \text{ mg ha}^{-1}$ ) (Table 3-12). Hybrid B104/CML323 had a low grain texture rating (1.7), the best plant appearance rating (1.2), and above average grain yield ( $3.2 \text{ mg ha}^{-1}$ ) (Table 3-12). Tropical hybrid CML323/CML288 had the best rating for grain texture (1.0), average rating for plant appearance (2.6) and below average grain yield ( $2.9 \text{ mg ha}^{-1}$ ) (Table 3-12). The three hybrids described previously were part of six hybrids in the experiment location which did not accumulate aflatoxin.

### *Weslaco 2002*

The mean aflatoxin accumulation for yellow hybrids at Weslaco in 2002 was  $160.00 \text{ ng g}^{-1}$  with a range of  $6.6 \text{ ng g}^{-1}$  to  $1093.5 \text{ ng g}^{-1}$  (Table 3-13). Significant differences were detected for aflatoxin, grain yield, grain texture, insect damage, anthesis date, silking date, anthesis-silking interval, plant height and ear height. No significant differences were detected for desirability and ear height to plant height ratio.

Table 3-12. Means and statistics for aflatoxin concentrations and secondary traits for yellow hybrids at College Station, TX in 2002.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	TXT 1 to 5	APP 1 to 5	GY mg ha <sup>-1</sup>
1	(LH235 x LH236) x CML285	29	9.9	3.2	2.7	2.9
2	(LH235 x LH236) x CML288	16	3.9	2.0	2.5	3.2
3	(LH235 x LH236) x CML323	30	25.9	2.0	2.7	2.9
4	FR2128 x NC300	10	1.9	2.5	2.1	3.3
5	(LH252 x LH262) x CML285	19	4.3	3.0	2.3	3.8
6	(LH252 x LH262) x CML288	22	4.7	1.7	2.8	3.2
7	(LH252 x LH262) x CML323	27	7.9	1.5	2.0	2.9
8	(LH252 x LH262) x NC300	4	0.0	3.0	2.2	3.2
9	B104 x CML285	13	3.3	3.0	2.8	3.1
10	B104 x CML288	24	5.3	2.0	2.2	3.1
11	B104 x CML323	3	0.0	1.7	1.2	3.2
12	B104 x NC300	26	7.1	2.7	3.5	2.4
13	Tx732 x CML323	7	1.2	2.0	1.8	3.0
14	CML285 x Tx732	17	4.0	4.0	2.4	3.1
15	CML338 x CML288	15	3.7	1.0	4.4	2.3
16	Tx732 x NC300	21	4.7	3.3	2.7	3.1
17	Tx770 x CML285	11	1.9	3.2	1.5	3.8
18	Tx770 x CML288	20	4.7	2.5	2.2	3.2
19	Tx770 x CML323	18	4.2	1.7	2.5	3.3
20	Tx714 x TX772	2	0.0	1.3	2.0	3.2
21	CML285 x CML323	25	6.4	2.2	2.6	3.0
22	NC300 x CML285	8	1.3	2.8	3.2	2.7
23	CML288 x CML285	6	0.0	2.1	2.9	2.8
24	NC300 x CML288	5	0.0	2.0	3.0	3.0
25	CML323 x CML288	1	0.0	1.0	2.6	2.9
26	CML323 x NC300	9	1.6	2.0	2.5	2.7
27	P31B13	12	3.2	3.0	3.1	3.5
28	P32R25	23	4.9	3.0	3.0	3.4
29	RX897	28	9.9	4.0	2.8	3.2
30	DK687	14	3.4	3.2	2.6	3.2
Mean			4.3	2.4	2.6	3.1
LSD <sup>§</sup>			.	0.7	1.2	0.7
Sig.			NS	***	**	*
C.V. % <sup>¶</sup>			150.2	20.0	32.9	16.3

\*, \*\* and \*\*\* Significant at .05, .01 and .001 levels, respectively, NS: Not Significant at .05

\*, \*\*, \*\*\* Significant at .05, .01, .001 level, respectively

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint to 5=dent), APP: Plant appearance (1=good plant appearance to 5=bad plant appearance), GY: Grain Yield (mg ha<sup>-1</sup>)

<sup>§</sup>Fisher's least significant difference

<sup>¶</sup>C.V. %: Coefficient of Variation

Table 3-13. Means and statistics for aflatoxin concentration and secondary traits for yellow hybrid evaluation at Weslaco, TX in 2002.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	GY Mg ha <sup>-1</sup>	TXT 1 to 5	ID 1 to 5	DES <sup>‡</sup> 1 to 5	MF days	FF days	ASI days	PH in	EH in	Eh/Ph
1	(LH235 x LH236) x CML285	8	32.6 g-m <sup>§</sup>	3.8	4.0	2.5	3.1	77.1	80.3	-3.3	82.0	32.9	0.4
2	(LH235 x LH236) x CML288	10	32.1 h-m	3.7	1.5	2.5	3.0	77.7	80.7	-3.0	72.5	30.0	0.4
3	(LH235 x LH236) x CML323	20	104.3 b-i	4.8	2.0	3.0	1.7	72.9	74.9	-1.8	72.0	32.5	0.4
4	FR2128 x NC300	3	12.2 k-m	5.0	2.0	2.0	2.6	76.8	80.1	-3.3	76.0	29.2	0.4
5	(LH252 x LH262) x CML285	25	257.9 a-e	3.5	3.0	2.5	3.8	76.2	79.7	-3.5	72.0	33.4	0.5
6	(LH252 x LH262) x CML288	24	180.9 b-g	3.9	1.0	1.5	3.1	78.0	80.8	-3.0	72.0	27.7	0.4
7	(LH252 x LH262) x CML323	16	68.7 d-j	4.3	2.0	1.5	2.9	71.7	72.4	-0.8	72.5	27.7	0.4
8	(LH252 x LH262) x NC300	13	67.5 e-k	4.5	2.0	2.0	2.1	74.1	76.3	-2.0	77.5	30.4	0.4
9	B104 x CML285	7	26.8 i-m	4.0	2.0	2.5	3.6	78.0	81.0	-3.0	74.5	25.5	0.3
10	B104 x CML288	18	63.6 e-k	3.0	1.5	1.5	3.9	78.0	81.1	-3.0	69.5	27.0	0.4
11	B104 x CML323	29	460.3 a-c	3.8	2.0	2.5	2.6	73.8	76.1	-2.0	70.5	29.5	0.4
12	B104 x NC300	4	12.5 j-m	3.2	2.0	2.0	3.6	76.2	79.7	-3.5	66.0	24.6	0.4
13	Tx732 x CML323	26	379.8 a-d	4.7	2.0	3.0	2.3	72.8	72.8	0.0	72.0	28.8	0.4
14	CML285 x Tx732	23	244.1 a-e	4.0	3.0	2.5	3.2	76.9	80.0	-3.3	68.0	27.4	0.4
15	CML338 x CML288	9	28.5 i-m	3.6	1.5	2.0	2.9	75.5	79.3	-3.8	73.0	28.6	0.4
16	Tx732 x NC300	21	175.3 b-h	4.6	3.0	3.0	2.9	73.8	77.7	-4.0	66.5	28.5	0.4
17	Tx770 x CML285	17	76.7 d-i	3.5	2.5	1.5	3.3	78.1	80.9	-3.0	74.5	35.2	0.5
18	Tx770 x CML288	5	20.5 i-m	3.7	2.0	3.0	4.6	77.9	80.9	-3.0	73.0	30.4	0.4
19	Tx770 x CML323	14	75.4 d-i	4.4	2.0	2.5	2.9	73.6	75.8	-2.0	66.5	33.9	0.5
20	Tx714 x TX772	22	210.6 a-f	4.5	2.0	2.5	2.4	74.2	76.9	-3.0	69.0	32.6	0.5
21	CML285 x CML323	11	38.2 f-l	3.8	1.5	1.5	1.9	78.4	81.2	-3.0	73.5	30.4	0.4
22	NC300 x CML285	19	93.0 c-i	4.0	2.5	3.0	3.4	78.5	81.4	-3.0	75.5	32.8	0.4

Table 3-13. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	GY Mg ha <sup>-1</sup>	TXT 1 to 5	ID 1 to 5	DES <sup>‡</sup> 1 to 5	MF days	FF days	ASI days	PH in	EH in	Eh/Ph
23	CML288 x CML285	12	63.3 e-k	2.8	2.0	2.0	4.6	78.1	81.0	-3.0	67.0	30.6	0.5
24	NC300 x CML288	6	30.6 i-m	3.4	2.0	1.0	4.1	76.6	80.0	-3.3	67.0	26.2	0.4
25	CML323 x CML288	1	6.6 m	3.5	1.0	1.5	4.1	76.0	79.4	-3.5	65.0	27.2	0.4
26	CML323 x NC300	2	7.6 l,m	3.7	2.0	2.0	2.6	73.6	78.2	-4.5	69.0	26.5	0.4
27	P31B13	30	1093.5 a	5.9	2.0	3.5	2.5	78.0	80.9	-3.0	75.0	31.5	0.4
28	P32R25	27	308.5 a-e	5.3	2.5	4.0	3.1	77.8	80.9	-3.0	75.5	36.2	0.5
29	RX897	15	85.9 c-i	5.5	4.0	3.5	2.8	74.8	76.6	-1.8	71.0	30.9	0.4
30	DK687	28	542.4 a,b	4.1	2.0	3.0	4.3	74.8	78.7	-3.8	73.5	30.2	0.4
	Mean		160.0	4.1	2.2	2.4	3.1	76.0	78.9	-2.9	71.7	30.0	0.4
	LSD <sup>¶</sup>		.	0.9	0.5	0.8	1.2	1.8	2.0	1.6	4.7	4.1	0.1
	Sig.		***	***	***	***	NS	***	***	**	*	*	NS
	C.V. % <sup>#</sup>		28.4	17.3	15.0	23.2	27.4	1.8	1.8	40.5	4.7	9.6	9.5

\*, \*\*, \*\*\* Significant at .05, .01, and .001 Levels, respectively, NS: Not significant at .05 level

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, GY: whole shelled plot weight converted to Mg ha<sup>-1</sup>, TXT: Grain Texture (1=flint to 5=dent), ID: insect damage (1=no ears damaged to 5=all ears damaged), Des: desirability (1=desirable plant type to 5=not desirable plant type), MF: days from planting to anthesis, FF: days from planting to silking, ASI: anthesis silking interval (FF-MF), PH: height of plant measured from ground to top of tassel, EH: height from ground to attachment of ear shank, Eh/Ph: ratio of height of ear to height of plant

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

Subtropical line CML323 and line NC300 were part of the three hybrids less susceptible for aflatoxin. Line CML323 crossed with tropical line CML288 was the least susceptible hybrid. Line CML323 crossed with line NC300 was the second least susceptible. Temperate line FR2128 crossed with line NC300 was the third least susceptible.

Hybrid CML323/CML288 had below average grain yield ( $3.5 \text{ mg ha}^{-1}$ ), one of the lowest grain texture rating (1.0), and a low rating for insect damage (1.5) (Table 3-13). Hybrid CML323/NC300 had a high grain yield ( $5.0 \text{ Mg ha}^{-1}$ ), a below average grain texture rating (2.0), and above average insect damage rating (3.0) (Table 3-13). Hybrid FR2128/NC300 had a high grain yield ( $5.0 \text{ Mg ha}^{-1}$ ), lower than average grain texture rating (2.0), low insect damage rating (2.0), and a lower than average desirability rating (2.6) (Table 3-13).

In addition of having lower accumulations, good grain yields were obtained by two of the three less susceptible hybrids. Grain yields of hybrids CML323/NC300 and FR2128/NC300 were not statistically different from those of commercial hybrid P31B13 ( $5.9 \text{ Mg ha}^{-1}$ ), which had the highest accumulation of aflatoxin ( $1093.5 \text{ ng g}^{-1}$ ).

#### *Corpus Christi 2002*

The mean aflatoxin accumulation for yellow hybrids at Corpus Christi in 2002 was  $839.0 \text{ ng g}^{-1}$  with a range of  $112.2 \text{ ng g}^{-1}$  to  $2418.2 \text{ ng g}^{-1}$  (Table 3-14). Significant differences were detected for aflatoxin accumulation.

Temperate line FR2128 crossed with line NC300 was the least susceptible hybrid, accumulating  $112.2 \text{ ng g}^{-1}$  aflatoxin (Table 3-14). Dekalb brand hybrid DK687

Table 3-14. Means and statistics for aflatoxin at Corpus Christi, TX in 2002

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>
1	(LH235 x LH236) x CML285	24	1433.8 a-c <sup>§</sup>
2	(LH235 x LH236) x CML288	7	284.4 d-g
3	(LH235 x LH236) x CML323	8	284.4 d-g
4	FR2128 x NC300	1	112.2 g
5	(LH252 x LH262) x CML285	27	1949.8 a,b
6	(LH252 x LH262) x CML288	10	413.5 c-g
7	(LH252 x LH262) x CML323	26	1586.7 a-c
8	(LH252 x LH262) x NC300	13	555.9 b-g
9	B104 x CML285	11	427.6 c-g
10	B104 x CML288	18	860.0 a-e
11	B104 x CML323	30	2418.2 a
12	B104 x NC300	12	434.5 c-g
13	Tx732 x CML323	28	1974.7 a,b
14	CML285 x Tx732	22	967.2 a-d
15	CML338 x CML288	9	395.4 c-g
16	Tx732 x NC300	6	229.6 d-g
17	Tx770 x CML285	17	703.1 a-f
18	Tx770 x CML288	5	228.0 d-g
19	Tx770 x CML323	25	1532.9 a-c
20	Tx714 x TX772	21	893.3 a-d
21	CML285 x CML323	19	862.0 a-e
22	NC300 x CML285	23	1015.1 a-d
23	CML288 x CML285	3	197.5 e-g
24	NC300 x CML288	4	198.8 e-g
25	CML323 x CML288	20	862.0 a-e
26	CML323 x NC300	14	599.8 a-f
27	P31B13	29	2275.1 a
28	P32R25	16	681.6 a-f
29	RX897	15	619.4 a-f
30	DK687	2	174.8 f,g
Mean			839.0
LSD <sup>¶</sup>			.
Sig.			***
C.V. % <sup>#</sup>			10.1

\*\*\* Significant at .001 Level

<sup>†</sup>Rank of genotypes by Aflatoxin concentration<sup>‡</sup> AF: antilogarithmic transformation of data<sup>§</sup>Mean separations determined using the logarithmic transformation of the data<sup>¶</sup>Fisher's least significant difference<sup>#</sup>C.V. %: Coefficient of Variation

accumulated the second lowest aflatoxin ( $174.8 \text{ ng g}^{-1}$ ) (Table 3-14). Tropical line CML288 crossed with tropical line CML285 accumulated the third lowest aflatoxin ( $197.5 \text{ ng g}^{-1}$ ) (Table 3-14).

#### *Across Locations 2002*

Twenty nine hybrids were combined for across location analysis in 2002. No significant differences were detected across locations for aflatoxin accumulation. The mean for hybrids across three locations was  $39.7 \text{ ng g}^{-1}$  with a range of  $8.2 \text{ ng g}^{-1}$  to  $122.2 \text{ ng g}^{-1}$  (Table 3-15). Subtropical and tropical lines CML 323, CML288 and NC300 were part of three hybrids less susceptible for aflatoxin contamination. Hybrid CML323/CML288 had the least accumulation of aflatoxin ( $8.2 \text{ ng g}^{-1}$ ). Temperate line FR2128 crossed with NC300 accumulated the second least aflatoxin ( $8.9 \text{ ng g}^{-1}$ ). Hybrid NC300/CML288 had the third least accumulation of aflatoxin ( $11.3 \text{ ng g}^{-1}$ ). Secondary traits were not included in the across location analysis because the traits were not evaluated at all three locations.

BLUPs change ranks from means in the across analysis of aflatoxin during 2002. The most noticeable rank change is with hybrid CML232/NC300, Changing from an aflatoxin accumulation mean rank of 3 to a BLUP ranking of 13 (Table 3-15).

The three locations in 2002 discriminated differently the response to aflatoxin hybrids. Singular value decomposition biplot shows that a strong response to environment is present, in that there are no large groupings for one environment (Figure 3-4).



Table 3-15. Means and statistics for aflatoxin concentration of yellow hybrids across locations in 2002.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>†</sup>	
			Mean	BLUP <sup>§</sup>
			ng g <sup>-1</sup>	ng g <sup>-1</sup>
1	(LH235 x LH236) x CML285	17	43.1	35.8
2	(LH235 x LH236) x CML288	11	21.4	29.9
3	(LH235 x LH236) x CML323	28	73.0	39.6
4	FR2128 x NC300	2	8.9	24.2
5	(LH252 x LH262) x CML285	29	75.1	40.8
6	(LH252 x LH262) x CML288	20	49.7	36.4
7	(LH252 x LH262) x CML323	23	54.3	37.8
8	(LH252 x LH262) x NC300	7	19.1	29.4
9	B104 x CML285	8	20.2	29.7
10	B104 x CML288	16	39.7	34.9
11	B104 x CML323	24	55.2	38.2
12	B104 x NC300	9	20.3	29.7
13	Tx732 x CML323	21	52.6	37.6
14	CML285 x Tx732	26	62.0	38.7
15	CML338 x CML288	10	21.3	30.0
16	Tx732 x NC300	19	43.6	35.1
17	Tx770 x CML285	12	27.0	31.9
18	Tx770 x CML288	6	18.4	28.8
19	Tx770 x CML323	18	43.5	35.9
20	Tx714 x TX772	14	33.1	33.5
21	CML285 x CML323	15	34.9	33.9
22	NC300 x CML285	13	27.3	32.1
23	CML288 x CML285	5	15.1	27.5
24	NC300 x CML288	4	11.3	25.7
25	CML323 x CML288	1	8.2	24.4
26	CML323 x NC300	3	9.7	33.4
27	P31B13	30	122.2	45.7
28	P32R25	27	68.9	39.5
29	RX897	22	53.8	37.3
30	DK687	25	57.1	37.2
Mean			39.7	33.8
Gen. Sig.			NS	
Env. Sig.			***	
Gen.*Env. Sig.			*	
C.V. % <sup>¶</sup>			40.5	

\* and \*\*\* Significant at .05 and .001 levels, respectfully, NS: Non significant at .05 level

†AF: antilogarithmic transformation of data

‡Rank of genotypes by Aflatoxin concentration

§Best Linear Unbiased Predictor

¶ C.V. %: Coefficient of Variation

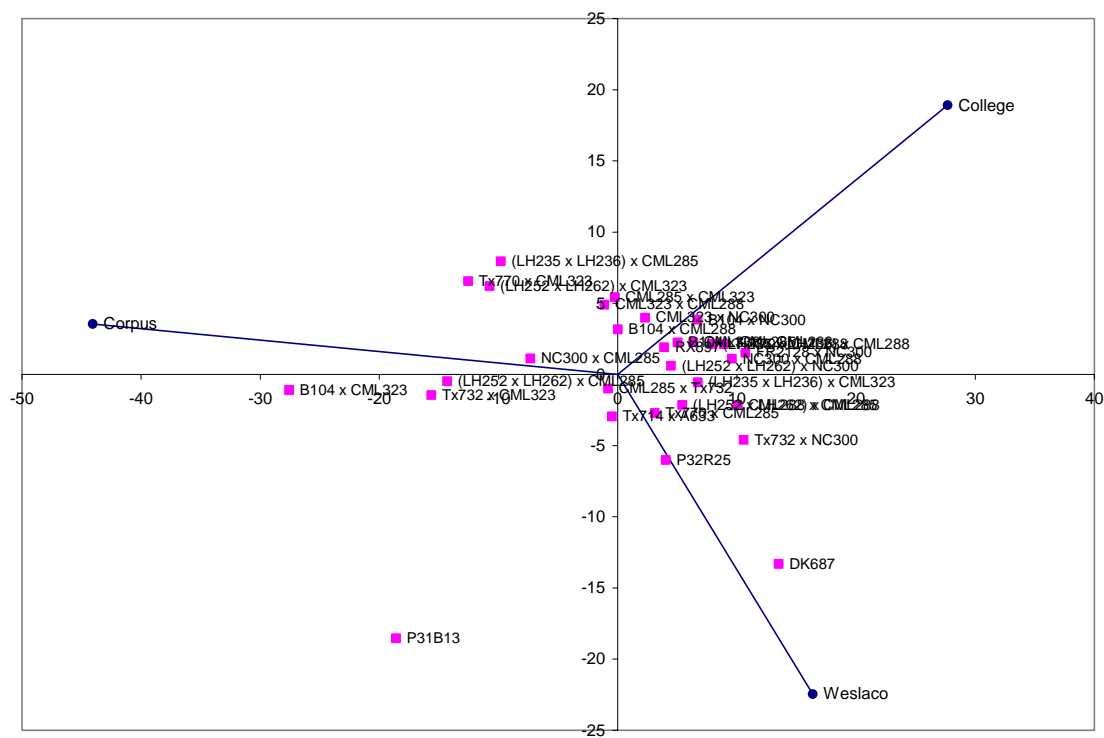


Figure 3-4. Singular value decomposition biplot of aflatoxin concentration at three Texas locations in 2002.

*College Station 2003*

The mean aflatoxin accumulation in yellow hybrids at College Station in 2003 was  $127.3 \text{ ng g}^{-1}$  with a range of  $6.2 \text{ ng g}^{-1}$  to  $429.0 \text{ ng g}^{-1}$  (Table 3-16). Significant differences were detected for aflatoxin, silking date, plant height, ear height, ear to plant ratio, grain texture, grain yield and test weight. Differences were not detected for lodging, kernel integrity and grain moisture.

Line Tx772 was part of three hybrids less susceptible for aflatoxin accumulation. Tropical line CML338 crossed with Tx772 accumulated the least aflatoxin. Temperate line B104 crossed with Tx772 accumulated the second least aflatoxin. Temperate tester LH195RR crossed with Tx772 produced the third least aflatoxin.

Hybrid CML338/Tx772 had an average silking date (83.5 d), a below average grain texture rating (2.1), an above average kernel integrity rating (3.4), a good grain yield ( $6.0 \text{ Mg ha}^{-1}$ ), harvest grain moisture (13.9 %) and test weight ( $77.4 \text{ kg hl}^{-1}$ ) (Table 3-16). Hybrid B104/Tx772 had an average silking date (83.8 d), a below average grain texture rating (2.2), above average kernel integrity rating (3.7) and grain yield ( $5.6 \text{ Mg ha}^{-1}$ ), a below average grain moisture (15.2 %), and a high test weight ( $78 \text{ kg hl}^{-1}$ ) (Table 3-16). Hybrid LH195RR/Tx772 had an above average silking date (85.1 d), an average grain texture rating (2.5), below average kernel integrity rating (2.9), above average grain yield ( $5.7 \text{ Mg ha}^{-1}$ ), an average grain moisture (15.4 %), and a high test weight ( $76.9 \text{ kg hl}^{-1}$ ) (Table 3-16).

Table 3-16. Means and statistics for aflatoxin concentrations and secondary traits for yellow hybrid evaluation at College Station, TX in 2003.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	PH	EH	EPR	LD	TXT	KI	GY	MS	TW
				d	cm	cm		1 to 5	1 to 5	1 to 5	Mg ha <sup>-1</sup>	%	kg hl <sup>-1</sup>
1	CML288/Tx714	9	79.9 d-k <sup>§</sup>	85.1	257.5	107.0	0.4	3.2	2.8	4.1	4.6	15.6	81.5
2	CML288/Tx772	4	16.5 l-n	83.3	236.2	94.0	0.4	2.1	2.2	3.4	5.1	14.0	76.4
3	Tx759/CML288	41	78.1 d-l	85.6	251.1	101.9	0.4	2.0	2.1	4.1	6.0	13.7	76.1
4	Tx770/CML288	42	416.9 a-c	85.1	280.3	118.4	0.4	2.2	2.7	3.1	6.1	14.4	74.7
5	CML323/Tx714	43	242.7 a-f	79.7	254.7	97.0	0.4	3.7	2.7	3.7	4.4	15.9	77.1
6	CML323/Tx732	40	161.3 a-g	83.1	228.3	89.2	0.4	3.7	2.4	3.7	5.8	14.2	75.5
7	CML323/Tx745	25	98.3 a-j	79.6	227.0	76.5	0.3	3.2	1.9	2.7	5.1	15.1	75.3
8	Tx770/CML323	17	100.0 a-j	83.1	254.8	107.2	0.4	3.2	2.6	4.1	5.5	15.5	78.0
9	B104/Tx772	2	9.3 m,n	83.8	272.6	103.0	0.4	2.6	2.2	3.7	5.6	15.2	78.0
10	CML285/Tx772	13	40.3 h-m	83.1	265.8	103.0	0.4	2.1	2.4	3.2	7.9	14.7	77.3
11	CML338/Tx772	1	6.2 n	83.5	247.0	113.7	0.5	2.3	2.1	3.4	6.0	13.9	77.4
12	Tx714/Tx772	19	70.8 d-l	84.0	258.1	107.2	0.4	2.7	2.8	1.9	7.0	14.1	73.7
13	Tx770/Tx772	18	88.6 c-k	82.7	241.1	95.3	0.4	1.9	2.4	5.0	5.8	16.1	77.3
14	CML327/NC300	34	156.7 a-g	83.8	273.1	116.8	0.4	3.5	3.3	3.4	6.6	14.8	74.3
15	NC300/CML285	24	114.2 a-j	87.8	276.0	117.4	0.4	4.1	3.6	3.1	7.2	17.2	77.2
16	NC300/CML288	16	68.4 d-l	85.3	248.9	100.3	0.4	3.6	2.8	2.8	6.9	14.8	74.1
17	NC300/Tx732	33	151.4 a-g	83.8	242.6	89.5	0.4	2.4	2.7	3.9	6.1	15.3	73.0
18	NC300/Tx745	6	30.5 i-m	83.0	260.6	96.2	0.4	3.2	2.8	3.8	6.7	14.6	71.3
19	Tx732/Tx770	37	252.6 a-e	84.5	247.7	104.8	0.4	2.6	3.2	3.9	6.5	14.3	74.5
20	Tx770/CML285	27	127.4 a-h	86.8	288.3	119.4	0.4	2.8	3.8	3.3	7.0	14.2	74.4
21	Tx770/CML338	20	46.8 g-l	84.1	257.5	112.1	0.4	3.5	2.6	2.9	5.6	14.7	74.6
22	Tx770/NC300	11	52.8 f-l	85.4	282.7	115.7	0.4	2.7	3.4	2.8	6.9	15.7	74.3
23	Tx770/Tx745	12	42.2 g-m	83.3	257.2	107.2	0.4	2.6	2.9	3.2	5.2	16.2	73.9

Table 3-16. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF	PH	EH	EPR	LD	TXT	KI	GY	MS	TW
				d	cm	cm		1 to 5	1 to 5	1 to 5	Mg ha <sup>-1</sup>	%	kg hl <sup>-1</sup>
24	FR2128/CML285	29	102.3 a-j	86.1	249.9	96.9	0.4	4.2	2.8	3.4	6.0	15.0	74.2
25	FR2128/CML288	36	169.8 a-g	86.1	268.9	115.9	0.4	4.0	2.3	3.1	7.0	14.7	75.0
26	FR2128/CML327	35	286.7 a-d	81.7	274.2	116.5	0.4	2.9	2.7	4.0	7.2	13.7	74.3
27	CML325/CML288	5	36.5 h-m	84.6	235.9	98.1	0.4	3.0	1.7	3.6	4.2	14.3	75.6
28	CML325/Tx770	32	112.2 a-j	82.7	253.0	99.6	0.4	3.6	2.4	2.6	5.1	15.6	77.6
29	CML338/NC300	10	40.0 h-m	84.0	254.0	106.0	0.4	3.3	2.8	3.3	6.8	14.1	74.8
30	CML338/Tx714	14	53.7 e-l	83.5	287.7	127.6	0.4	2.4	2.9	3.1	7.2	14.1	75.3
31	CML338/Tx732	30	170.8 a-g	82.8	242.9	92.0	0.4	2.6	2.9	4.2	6.2	15.3	75.3
32	CML338/Tx745	31	151.4 a-g	84.1	255.6	101.3	0.4	2.6	2.7	3.1	4.9	15.4	76.0
33	CML323/CML288	23	83.2 d-k	83.3	253.0	101.3	0.4	2.9	1.9	2.9	3.7	17.1	78.2
34	FR2128/CML323	8	52.8 f-l	82.1	238.4	91.8	0.4	2.2	1.9	3.1	6.4	14.1	76.8
35	FR2128/NC300	38	230.4 a-f	84.6	258.7	105.7	0.4	4.0	3.0	3.0	6.4	14.7	73.0
36	SCR42/Tx772	22	92.8 a-j	85.0	258.4	94.0	0.4	2.1	2.1	3.4	6.4	14.3	78.1
37	LH195RR/Tx772	3	20.3 k-n	85.1	241.2	87.8	0.4	1.9	2.5	2.9	5.7	15.4	76.9
38	LH310RR/Tx772	7	26.8 i-m	85.5	252.1	85.7	0.3	1.9	2.2	3.4	5.5	13.9	75.4
39	Pop. 69 Templado Amarillo QPM-B-B-B3-5/TX804	21	89.1 c-k	81.8	264.2	108.0	0.4	3.3	2.9	4.4	5.5	16.8	77.6
40	Pop. 69 Templado Amarillo QPM-B-B-B4-7/TX804	44	441.6 a	85.0	253.8	106.3	0.4	3.1	2.6	5.6	5.0	14.7	76.7
41	P31B13	39	300.3 a-d	85.6	238.4	104.5	0.4	1.2	3.1	4.5	7.7	13.6	74.3
42	P32R25	45	429.0 a,b	83.1	275.3	107.0	0.4	2.5	3.5	4.9	7.5	12.5	72.6
43	LH195/LH210	15	65.3 d-l	84.0	261.5	108.0	0.4	2.1	3.9	4.2	7.8	14.7	73.6
44	RX897	26	191.6 a-g	83.4	250.6	103.9	0.4	6.1	4.2	3.3	6.8	13.4	72.8
45	DK668	28	131.8 a-h	83.5	255.3	97.2	0.4	2.1	3.6	3.6	7.6	13.9	73.9

Table 3-16. Continued

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup>	FF d	PH cm	EH cm	EPR	LD 1 to 5	TXT 1 to 5	KI 1 to 5	GY Mg ha <sup>-1</sup>	MS %	TW kg hl <sup>-1</sup>
	Mean		127.3	83.9	256.6	103.4	0.4	2.9	2.5	3.0	4.4	15.4	81.1
	LSD <sup>¶</sup>			2.0	21.2	15.4	0.0	1.9	0.8	1.8	1.9	2.7	3.4
	Sig.		***	***	***	**	***	NS	***	NS	***	NS	***
	C.V.,% <sup>#</sup>		24.9	1.7	5.9	10.7	8.5	47.7	20.4	54.2	25.9	10.5	2.5

\*\* and \*\*\* Significant at .01 and .001 Levels, respectively

NS: not significant at .05.

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, PH: height of plant measured from ground to top of tassel, EH: height from ground to attachment of ear shank, EPR: ratio of height of ear to height of plant, LD: Lodging (1=all plants standing erect to 5=all plants lodged), TXT: Grain Texture (1=flint to 5=dent), KI: kernel integrity (1=no kernels broken on ear to 5=majority of kernels broken on ear), GY: grain yield (Mg ha<sup>-1</sup>), MS: grain moisture at harvest, TW: test weight (kg hl<sup>-1</sup>).

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

*Weslaco 2003*

The mean aflatoxin accumulation for yellow hybrids at Weslaco in 2003 was 160.2 ng g<sup>-1</sup> with a range of 17.0 ng g<sup>-1</sup> to 1053.2 ng g<sup>-1</sup> (Table 3-17). Significant differences were detected for all traits measured.

Tropical line CML338 was part of two hybrids less susceptible for aflatoxin accumulation. Line CML338 crossed with Tx772 was the least susceptible hybrid. Line CML338 crossed with line NC300 was the second least susceptible hybrid. Line CML327 crossed with subtropical line NC300 was the third least susceptible hybrid.

Hybrid CML338/Tx772 had the lowest grain texture rating (1.0), the lowest kernel integrity rating (1.0) and a below average grain yield (3.6 Mg ha<sup>-1</sup>) (Table 3-17). Hybrid CML338/NC300 had a below average grain texture rating (2.1), a low kernel integrity rating (1.7) and a below average grain yield (3.7 Mg ha<sup>-1</sup>) (Table 3-17). Hybrid CML327/NC300 had a low anthesis silking interval (1.9 d), higher than average grain texture rating (2.9), an average kernel integrity rating (2.3), and lower than average grain yield (3.2 Mg ha<sup>-1</sup>) (Table 3-17). Line Tx772 tended to be more susceptible in Weslaco, than in College Station. Aflatoxin accumulations for Tx772 hybrids ranged from the best 17.0 ng g<sup>-1</sup> to the worst 1053.2 ng g<sup>-1</sup> (Table 3-17).

Table 3-17. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at Weslaco, TX in 2003.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	FF d	MF d	ASI d	TXT 1 to 5	KI 1 to 5	DES 1 to 5	GY Mg ha <sup>-1</sup>
1	CML288/Tx714	6	22.8 o-r <sup>§</sup>	66.1	67.0	1.1	2.0	1.6	1.9	3.8
2	CML288/Tx772	23	100.0 e-p	63.7	66.5	2.8	1.0	1.6	2.5	3.0
3	Tx759/CML288	27	118.9 c-m	65.2	68.0	2.7	2.0	2.8	3.7	2.9
4	Tx770/CML288	24	103.5 e-o	68.0	70.0	1.7	2.2	2.2	2.7	3.4
5	CML323/Tx714	19	59.9 g-r	62.5	65.3	2.8	2.2	2.3	1.7	4.4
6	CML323/Tx732	34	188.4 b-i	62.1	64.6	2.5	2.2	3.2	2.4	4.5
7	CML323/Tx745	22	82.7 f-q	66.2	67.5	1.2	1.5	1.7	1.7	4.8
8	Tx770/CML323	41	436.5 a-e	62.4	65.2	2.9	2.0	3.1	3.0	3.5
9	B104/Tx772	11	30.0 l-r	63.1	67.0	4.1	1.6	2.2	2.2	3.6
10	CML285/Tx772	45	1053.2 a	68.1	70.7	2.7	1.6	1.8	2.0	3.7
11	CML338/Tx772	1	17.0 r	60.5	63.7	2.9	1.0	1.2	1.9	3.6
12	Tx714/Tx772	29	134.9 c-l	63.8	68.3	4.4	2.0	2.2	2.0	4.1
13	Tx770/Tx772	37	237.1 a-h	64.7	67.1	2.6	1.9	2.0	2.5	3.8
14	CML327/NC300	3	20.5 q,r	63.9	65.7	1.9	2.9	2.3	2.6	3.2
15	NC300/CML285	30	163.1 b-k	68.1	70.2	2.3	3.1	2.1	2.2	3.8
16	NC300/CML288	21	73.7 f-r	65.3	68.5	3.2	2.1	2.3	3.0	2.3
17	NC300/Tx732	33	186.2 b-i	62.1	65.3	3.2	3.2	3.0	2.6	4.2
18	NC300/Tx745	15	49.0 i-r	64.7	67.0	2.6	2.0	1.6	1.3	5.2
19	Tx732/Tx770	35	201.8 b-i	65.2	68.5	3.1	3.4	3.3	3.0	4.2
20	Tx770/CML285	43	543.3 a-c	68.3	70.0	1.5	3.0	1.9	2.2	3.7
21	Tx770/CML338	4	20.8 q,r	64.6	68.5	4.1	2.0	1.7	2.3	4.7
22	Tx770/NC300	17	51.3 h-r	64.7	67.6	2.9	2.9	2.3	2.6	3.5
23	Tx770/Tx745	5	21.8 p-r	65.9	67.5	1.6	2.2	1.8	1.8	4.9
24	FR2128/CML285	40	348.7 a-f	65.8	68.0	2.4	2.0	1.5	1.5	4.5



Table 3-17. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF ng g <sup>-1</sup>	FF d	MF d	ASI d	TXT 1 to 5	KI 1 to 5	DES 1 to 5	GY Mg ha <sup>-1</sup>
25	FR2128/CML288	8	25.1 m-r	67.0	68.9	2.1	1.6	1.7	2.1	3.8
26	FR2128/CML327	42	492.6 a-d	65.8	67.0	1.0	2.0	2.5	2.7	3.7
27	CML325/CML288	12	34.7 k-r	64.9	67.8	3.1	1.0	1.9	3.0	3.5
28	CML325/Tx770	32	165.0 b-j	63.1	66.0	2.9	2.0	2.4	2.6	3.9
29	CML338/NC300	2	18.3 q,r	61.6	63.8	2.2	2.1	1.7	2.5	3.7
30	CML338/Tx714	36	213.8 b-i	61.2	63.6	2.4	2.1	2.1	1.7	4.4
31	CML338/Tx732	18	56.6 g-r	61.0	63.7	2.8	2.5	1.9	2.6	3.9
32	CML338/Tx745	7	23.2 n-r	62.5	65.3	2.7	1.8	1.7	1.5	4.6
33	CML323/CML288	10	27.1 m-r	63.3	65.8	2.6	1.0	1.5	2.0	4.1
34	FR2128/CML323	25	104.1 d-o	62.1	64.5	2.6	1.2	2.1	2.2	3.8
35	FR2128/NC300	9	26.2 m-r	64.4	67.9	3.6	2.5	2.4	2.1	4.1
36	SCR42/Tx772	31	164.1 b-k	67.0	69.0	2.1	1.2	2.2	2.3	3.4
37	LH195RR/Tx772	13	35.9 j-r	62.2	66.4	3.8	1.9	2.3	2.5	3.4
38	LH310RR/Tx772	20	63.1 g-r	63.5	65.5	2.1	1.6	1.9	1.7	4.0
39	Pop. 69 Templado Amarillo QPM-B-B-B3-5/TX804	44	649.4 a,b	62.5	65.7	3.3	3.0	4.2	3.4	4.0
40	Pop. 69 Templado Amarillo QPM-B-B-B4-7/TX804	38	251.2 a-g	62.4	65.2	2.9	3.3	4.3	3.8	3.7
41	P31B13	39	252.6 a-g	66.2	67.9	1.4	2.5	2.6	1.6	5.5
42	P32R25	28	133.4 c-l	66.8	69.2	2.1	2.3	3.2	2.5	4.5
43	LH195 x LH210	26	109.6 d-n	62.8	65.9	2.8	3.5	2.1	1.1	6.2
44	RX897	14	46.5 i-r	64.5	66.5	2.0	3.8	2.6	1.7	5.6
45	DK668	16	50.7 h-r	63.3	65.3	2.0	3.1	2.5	2.3	5.4

Table 3-17. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF	FF	MF	ASI	TXT	KI	DES	GY
			ng g <sup>-1</sup>	d	d	d	1 to 5	1 to 5	1 to 5	Mg ha <sup>-1</sup>
Mean			160.2	64.3	66.9	2.6	2.2	2.3	2.3	4.1
LSD <sup>¶</sup>				2.1	2.4	1.5	0.4	0.8	0.8	0.9
Sig.			***	***	***	***	***	***	***	***
C.V.% <sup>#</sup>			24.8	2.3	2.5	41.3	12.3	24.2	25.3	15.9

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, MF: days from planting to anthesis, ASI: anthesis silking interval (silking date-anthesis date), TXT: Grain Texture (1=flint low 5=dent), KI: kernel integrity (1=no kernels broken on ear to 5=majority of kernels broken on ear), Des: desirability (1=desirable plant type to 5=not desirable plant type), GY: grain yield (Mg ha<sup>-1</sup>).

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

*Corpus Christi 2003*

The mean aflatoxin accumulation for yellow hybrids at Corpus Christi in 2003 was  $26.4 \text{ ng g}^{-1}$  with a range of  $3.0 \text{ ng g}^{-1}$  to  $140.4 \text{ ng g}^{-1}$  (Table 3-18). Significant differences were detected for all traits measured.

Tropical line CML285 was part of three hybrids less susceptible for aflatoxin accumulation. Line NC300 crossed with CML285 accumulated the least aflatoxin. Line CML285 crossed with Tx772 accumulated the second least. Temperate line FR2128 crossed with CML285 produced the third hybrid less susceptible for aflatoxin.

Hybrid NC300/CML285 had a lower than average kernel integrity rating (1.9), higher than average grain texture rating (2.9), and higher than average desirability rating (2.4) (Table 3-18). Hybrid CML285/Tx772 had a lower than average kernel integrity rating (1.9), a tie for the lowest grain texture rating (1.0), and the highest desirability rating (3.6) (Table 3-18). Hybrid FR2128/CML285 had a lower than average kernel integrity rating (1.9), a higher than average grain texture rating (2.1), and desirability rating (2.1) (Table 3-18). The only other hybrid with CML285 (Tx770/CML285) did not statistically differ from the three hybrids for aflatoxin accumulation. Hybrid Tx770/CML285, however, scored average or higher than average rankings on all secondary traits.

Table 3-18. Means and statistics of aflatoxin and secondary traits for yellow hybrids at Corpus Christi, TX in 2003.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	KI 1 to 5	TXT 1 to 5	DES 1 to 5
1	CML288/Tx714	33	42.9 a-h <sup>§</sup>	2.1	2.0	2.2
2	CML288/Tx772	14	10.2 f-l	1.6	1.0	2.2
3	Tx759/CML288	44	78.1 a,b	2.0	1.6	1.9
4	Tx770/CML288	32	23.0 b-i	2.3	2.5	2.1
5	CML323/Tx714	34	37.4 a-h	2.4	2.0	2.2
6	CML323/Tx732	45	140.4a	3.4	2.0	2.6
7	CML323/Tx745	37	14.2 c-l	2.1	1.1	2.0
8	Tx770/CML323	42	57.5 a-d	2.6	2.0	3.1
9	B104/Tx772	7	9.6 f-l	3.1	1.3	3.3
10	CML285/Tx772	2	3.4 k-m	1.6	1.3	1.9
11	CML338/Tx772	8	6.0 i-m	1.9	1.0	3.6
12	Tx714/Tx772	22	14.2 c-l	2.8	1.8	2.5
13	Tx770/Tx772	20	18.6 b-j	2.5	1.3	3.4
14	CML327/NC300	5	1.8 m	2.0	2.6	2.2
15	NC300/CML285	1	3.0 l,m	1.9	2.9	2.4
16	NC300/CML288	4	3.7 j-m	1.5	1.9	1.8
17	NC300/Tx732	23	16.1 b-l	1.9	2.4	1.9
18	NC300/Tx745	19	9.2 f-m	2.3	2.0	1.5
19	Tx732/Tx770	43	71.6 a-c	3.3	3.0	3.2
20	Tx770/CML285	6	9.0 f-m	2.1	3.6	2.7
21	Tx770/CML338	35	47.9 a-f	2.4	2.0	2.4
22	Tx770/NC300	11	11.1 d-l	3.0	3.0	3.2
23	Tx770/Tx745	16	8.9 g-m	2.5	2.4	2.5
24	FR2128/CML285	3	5.2 i-m	1.9	2.1	2.1
25	FR2128/CML288	18	9.4 f-m	1.6	1.6	2.1
26	FR2128/CML327	41	42.9 a-h	2.5	2.0	1.8
27	CML325/CML288	15	12.1 d-l	1.6	1.0	1.7
28	CML325/Tx770	40	66.8 a-c	2.4	1.9	2.2
29	CML338/NC300	9	8.1 h-m	1.7	2.0	2.8
30	CML338/Tx714	21	10.7 e-l	2.2	1.8	2.0
31	CML338/Tx732	30	44.4 a-g	2.2	2.0	2.6
32	CML338/Tx745	31	26.0 b-i	2.5	2.0	2.3
33	CML323/CML288	13	10.0 f-l	1.4	1.0	2.7
34	FR2128/CML323	17	8.3 g-m	3.3	3.7	2.7
35	FR2128/NC300	10	15.6 b-l	2.1	2.0	1.9
36	SCR42/Tx772	26	16.9 b-k	2.5	1.1	2.3
37	LH195RR/Tx772	12	9.1 f-m	2.1	1.3	2.4
38	LH310RR/Tx772	24	12.2 d-l	2.3	1.4	1.9
39	Pop. 69-B-B-B3-5/TX804	28	27.7 a-i	3.8	1.9	3.3

Table 3-18. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF	KI	TXT	DES
			ng g <sup>-1</sup>	1 to 5	1 to 5	1 to 5
40	Pop. 69-B-B-B4-7/TX804	39	43.9 a-g	3.8	2.0	3.5
41	P31B13	38	42.4 a-h	3.4	2.1	1.9
42	P32R25	27	19.1 b-j	3.6	2.3	2.6
43	LH195 x LH210	36	56.9 a-e	3.5	3.4	3.0
44	RX897	25	22.4 b-i	2.7	3.5	2.3
45	DK668	29	39.1 a-h	3.1	3.5	2.0
	Mean		26.4	2.2	2.0	1.5
	LSD <sup>¶</sup>		.	0.7	0.41	0.7
	Sig		***	***	***	***
	C.V. % <sup>#</sup>		42.4	22.6	14.4	31.6

\*\*\* Significant at .001 Level

<sup>†</sup>Rank of genotypes by Aflatoxin concentration<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, KI: Kernel Integrity (1=no kernels broken to 5=majority of kernels damaged), TXT: Grain Texture (1=flint to 5=dent), Des: Plant desirability (1=desirable to 5=undesirable)<sup>§</sup>Mean separations determined using the logarithmic transformation of the data<sup>¶</sup>Fisher's least significant difference, used to compare individual genotypes<sup>#</sup>C.V. %: Coefficient of Variation*Across Locations 2003*

Data for forty four hybrids and three locations were combined for across location analysis and BLUP estimated for aflatoxin and grain texture. Significant differences were detected for both traits (aflatoxin and grain texture) for genotype, environment and genotype\*environment interaction in the combined analysis. The mean aflatoxin accumulation across environments in 2003 was 64.6 ng g<sup>-1</sup> with a range of 8.6 ng g<sup>-1</sup> to 182.3 ng g<sup>-1</sup> (Table 3-19).

Line Tx772 is a parent of two hybrids less susceptible to aflatoxin accumulation across locations. CML338/Tx772 hybrids had the least aflatoxin accumulation (8.6 ng g<sup>-1</sup>) and lowest grain texture rating (1.0) across locations (Table 3-19). The hybrid B104/

Tx772 had the second lowest aflatoxin accumulation ( $13.9 \text{ ng g}^{-1}$ ) and a lower than average grain texture rating (1.4) across locations (Table 3-19). The subtropical hybrid CML327/NC300 was the third least susceptible hybrid for aflatoxin accumulation ( $17.9 \text{ ng g}^{-1}$ ) and higher than average grain texture rating (2.8) (Table 3-19).

BLUPs calculated for aflatoxin across locations showed higher estimates than that of the least squared means, however, the rankings of hybrids did not changed. The BLUPs calculated for grain texture vary only by a 0.1 difference in 9 hybrids.

For the hybrids included in the across location analysis, the mean accumulation at College Station was  $88.3 \text{ ng g}^{-1}$ , Corpus Christi was  $17.4 \text{ ng g}^{-1}$  and Weslaco was  $88.8 \text{ ng g}^{-1}$ . Corpus Christi was lower than previous years and studies due to the method of inoculation (i.e., placing colonized kernels on the ground between the plant rows) and unusually high rainfall just after inoculation time.

Singular value decomposition biplot for aflatoxin concentration across locations showed three distinctly different locations of hybrid response to aflatoxin (Figure 3-5). No clear grouping is apparent for experimental locations and response to aflatoxin accumulation during 2003.

A good example of the change in ranks seen in the genotype\*environment interaction is with hybrid CML327/NC300. Hybrid CML327/NC300 ranked number three for aflatoxin accumulation at Weslaco and ranked number five at Corpus Christi, while ranking 34 at College Station.

Figure 3-5 . Singular value decomposition biplot of aflatoxin concentration in yellow hybrids at three Texas locations in 2003.

Table 3-19. Means and statistics of aflatoxin and texture for yellow hybrids across three Texas locations in 2003.

Entry	Pedigree	Rank <sup>‡</sup>	AF <sup>†</sup>		TXT	
			Mean	BLUP <sup>§</sup>	Mean	BLUP
			ng g <sup>-1</sup>	ng g <sup>-1</sup>	1 to 5	1 to 5
1	CML288/Tx714	17	42.7 g-p <sup>§</sup>	46.3	2.0	2.0
2	CML288/Tx772	9	25.7 m-q	34.7	1.0	1.0
3	Tx759/CML288	34	89.8 a-h	70.5	1.8	1.7
4	Tx770/CML288	35	99.8 a-f	74.9	2.3	2.3
5	CML323/Tx714	32	81.6 a-j	66.8	2.1	2.1
6	CML323/Tx732	42	162.2 a,b	98.5	2.1	2.0
7	CML323/Tx745	20	48.7 e-n	49.9	1.3	1.3
8	Tx770/CML323	39	135.9 a-d	89.1	2.0	1.9
9	B104/Tx772	2	13.9 q,r	24.5	1.4	1.4
10	CML285/Tx772	23	52.3 e-m	51.9	1.5	1.5
11	CML338/Tx772	1	8.6 r	18.7	1.0	1.0
12	Tx714/Tx772	22	51.4 e-m	51.4	1.9	1.9
13	Tx770/Tx772	28	73.1 b-k	62.8	1.6	1.6
14	CML327/NC300	3	17.9 p-r	28.3	2.8	2.8
15	NC300/CML285	16	38.4 h-p	43.6	3.0	3.0
16	NC300/CML288	10	26.6 l-q	35.4	2.0	2.0
17	NC300/Tx732	31	76.9 a-j	64.6	2.7	2.6
18	NC300/Tx745	7	23.9 m-q	33.4	2.0	2.0
19	Tx732/Tx770	41	154.0 a-c	95.7	3.1	3.0
20	Tx770/CML285	33	85.4 a-i	68.6	3.5	3.4
21	Tx770/CML338	15	36.0 i-p	42.0	2.0	2.0
22	Tx770/NC300	13	31.1 k-q	38.7	2.9	2.9
23	Tx770/Tx745	6	20.1 n-r	30.2	2.3	2.3
24	FR2128/CML285	24	57.0 d-m	54.5	2.1	2.1
25	FR2128/CML288	14	34.2 j-q	40.9	1.7	1.7
26	FR2128/CML327	44	182.3 a	105.2	2.0	2.0
27	CML325/CML288	8	24.8 m-q	34.1	1.0	1.0
28	CML325/Tx770	37	107.3 a-f	78.0	1.9	1.9
29	CML338/NC300	4	18.1 p-r	28.5	2.1	2.1
30	CML338/Tx714	21	49.6 e-n	50.4	2.0	2.0
31	CML338/Tx732	30	75.4 a-k	63.9	2.2	2.2
32	CML338/Tx745	18	45.0 f-o	47.7	1.9	1.9
33	CML323/CML288	12	28.2 l-q	36.7	1.0	1.0
35	FR2128/NC300	19	45.4 f-o	48.0	2.3	2.2
36	SCR42/Tx772	26	63.6 c-l	58.0	1.2	1.2
37	LH195RR/Tx772	5	18.8 o-r	29.1	1.6	1.6
38	LH310RR/Tx772	11	27.4 l-q	36.0	1.5	1.5
39	Pop. 69-B-B-B3-5/TX804	38	117.0 a-e	81.9	2.4	2.3
40	Pop. 69-B-B-B4-7/TX804	43	169.5 a,b	101.0	2.5	2.4



Table 3-19. Continued.

Entry	Pedigree	Rank <sup>‡</sup>	<u>AF</u> <sup>†</sup>		<u>TXT</u>	
			Mean	BLUP	Mean	BLUP
			ng g <sup>-1</sup>	ng g <sup>-1</sup>	1 to 5	1 to 5
41	P31B13	40	147.6 a-c	93.4	2.4	2.4
42	P32R25	36	102.9 a-f	76.2	2.4	2.4
43	LH195 x LH210	29	74.1 a-k	63.3	3.5	3.5
44	RX897	25	58.4 d-m	55.3	3.7	3.7
45	DK668	27	63.9 c-l	58.2	3.4	3.3
	Mean		64.6	55.8	2.1	2.1
	LSD <sup>#</sup>		.		0.3	
	Gen. Sig.		***		***	
	Env. Sig.		***		***	
	Gen.*Env. Sig.		***		***	
	C.V.% <sup>††</sup>		28.6		13.6	

\*\*\* Significant at .001 Level

<sup>†</sup>Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint to 5=dent)

<sup>‡</sup>Rank of genotypes by Aflatoxin concentration

<sup>§</sup>Best Linear Unbiased Predictor

<sup>¶</sup>Mean separations determined using the logarithmic transformation of the data

<sup>#</sup>Fisher's least significant difference

<sup>††</sup>C.V. %: Coefficient of Variation

### *College Station 2004*

The mean aflatoxin accumulation for yellow hybrids at College Station in 2004 was 155.4 ng g<sup>-1</sup> with a range of 44.7 ng g<sup>-1</sup> to 1698.2 ng g<sup>-1</sup> (Table 3-20). Non significant differences were detected for aflatoxin and grain yield rating. Significant differences were detected for plant height, ear height, ear to plant ratio, grain texture, ear aspect and ear yield.

Two of the three least susceptible hybrids are derived from crosses made with tropical and temperate lines. QPM line derived from Population 69 crossed with LH195 accumulated the least aflatoxin. Line (Tx772/CML326)-B-B6-B-B-B crossed with

LH195 accumulated second least aflatoxin. Line (CML285/B104)-B-4-B-B-B-B crossed with LH195 accumulated the third least.

Hybrid Pop. 69-B-B-B1-8-B-B-B/LH195 had lower than average grain texture rating (1.7), higher than average ear aspect rating (3.3) and grain yield rating (3.5), and lower than average ear yield (113.7 g). Hybrid (Tx772/CML326)-B-B6-B-B-B/LH195 had lower than average grain texture rating (1.7), higher than average ear aspect rating (2.7), slightly higher than average grain yield rating (2.7), and a lower than average ear yield (115.2 g). Hybrid (CML285-B104)-B-4-B-B-B-B/LH195 had higher than average grain texture rating (2.7), lower than average ear aspect rating (1.8), lower than average grain yield rating (2.0), and the fifth highest ear yield (141.2 g).

Several of the parental lines of testing hybrids were derived from crosses among lines previously tested for aflatoxin (e.g., from Tx772). Some of the derived lines behaved as their parental lines and others did not in their capability to reduce aflatoxin concentration of hybrids. There are cases where two closely related lines derived from the same cross, (TX772/CML326)-B-B6-B-B-B (46.4 ng g<sup>-1</sup>) and (TX772/CML326)-B-B6-B-B (190.5 ng g<sup>-1</sup>), had contrasting responses to aflatoxin.

Several of the lines in hybrid combinations at College Station in 2004 had ear yields statistically similar to that of the hybrid checks (Table 3-20). Of those lines with hybrids of similar ear yield to the commercial hybrid checks, only one inbred's hybrid accumulated more aflatoxin than the commercial hybrids (Table 3-20).

Table 3-20. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at College Station, TX in 2004.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	PH cm	EH cm	EPP	TXT 1 to 5	EA 1 to 5	EY g ear <sup>-1</sup>
1	NC300/CML288-B-2-B-B-B/LH195	5	52.1	247.0	105.8	0.4	1.8	1.3	164.6
2	(CML 415/CML304)-B-2-1-B-B/LH195	7	94.3	258.4	112.9	0.4	1.7	1.8	139.1
3	(CML 326/TX772)-B-1-B-B-B-B/LH195	14	145.6	259.7	111.9	0.4	1.5	2.0	135.1
4	(CML 326/TX772)-B-11-B-B-B-B/LH195	12	131.8	246.6	102.9	0.4	1.8	2.5	122.1
5	(CML288/NC300)-B-3-B-B-B-B/LH195	6	83.8	233.0	97.9	0.4	2.0	1.7	137.7
6	(CML288/NC300)-B-9-B1-B-B-B/LH195	8	114.8	256.6	103.2	0.4	1.8	1.8	138.4
7	(NC300/TX772)-B-1-B2-B-B-B/LH195	19	204.2	247.0	104.8	0.4	2.2	3.0	117.6
8	(TX772/CML326)-B-B5-B-B-B/LH195	15	150.2	263.8	122.5	0.5	1.7	2.0	132.1
9	(TX772/CML326)-B-B6-B-B/LH195	18	190.5	246.1	105.6	0.4	1.5	2.3	128.7
10	(TX772/CML326)-B-B6-B-B-B/LH195	2	46.4	255.2	111.3	0.4	1.7	2.7	115.2
11	(CML269/Tx110)-B-2-B-B-B-B/LH195	9	118.4	253.7	104.6	0.4	2.7	2.2	123.8
12	Pop. 69-B-B-B1-6-B-B-B/LH195	16	158.5	242.8	115.0	0.5	1.5	2.3	131.6
13	Pop. 69-B-B-B1-8-B-B-B/LH195	1	44.7	241.2	116.8	0.5	1.7	3.3	113.7
14	(CML285/B104)-B-4-B-B-B-B/LH210	3	49.4	253.3	119.2	0.5	2.7	1.8	141.2
15	Pop. 69-B-B-B2-11-B-B-B/LH195	11	130.8	257.5	115.8	0.5	1.7	3.0	123.8
16	Pop. 69-B-B-B4-1-B-B-B/LH195	20	212.2	266.6	117.0	0.4	1.7	2.5	132.1
17	Pop. 69-B-B-B4-7-B-B-B/LH195	28	433.2	250.3	114.5	0.5	1.3	2.8	132.8
18	CML289/Tx772-B-B-B-B-B/LH210	10	124.9	246.2	119.5	0.5	2.2	3.0	114.0
19	NC300/CML288-B-1-B-B-B/LH210	22	225.6	244.5	117.1	0.5	2.3	1.8	128.3
20	NC300/CML288-B-4-B-B-B/LH210	4	50.1	258.7	114.9	0.4	2.7	2.3	132.2
21	NC300/CML288-B-5-B-B-B/LH210	24	244.2	242.7	119.5	0.5	2.0	2.2	138.9
22	Tx770/CML288-B-3-B-B-B/LH210	23	225.6	249.5	119.0	0.5	2.0	2.3	145.0
23	((CML 408/B104)x(CML 411/B104))-2-3-B-B/LH210	27	383.1	257.3	118.0	0.5	3.2	3.2	124.1
24	SCR42 x Tx772	17	189.1	247.7	98.6	0.4	1.0	1.7	133.8

Table 3-20. Continued.

Entry	Pedigree	Rank <sup>‡</sup>	AF <sup>‡</sup>	PH	EH	EPR	TXT	EA	EY
			ng g <sup>-1</sup>	cm	cm		1 to 5	1 to 5	g
25	(CML 408/B104)-B-2-1-B-B/LH210	13	132.8	253.8	113.5	0.5	3.0	2.0	135.9
26	DKC66-80	21	223.9	256.8	112.7	0.4	3.3	2.7	129.5
27	DKC69-70	26	374.4	265.7	118.8	0.4	2.0	1.2	152.6
28	P31B13	29	1698.2	225.4	115.2	0.5	2.3	3.3	142.5
29	P32R25	25	271.2	256.0	122.5	0.5	2.3	2.3	139.4
30	LH195 x LH210	30	.	270.1	127.4	0.5	2.8	2.8	.
	Mean		155.4	251.8	113.3	0.5	2.1	2.3	132.6
	LSD <sup>§</sup>		.	19.6	11.7	0.0	0.6	0.9	22.3
	Sig.		NS	**	***	***	***	***	**
	C.V.% <sup>¶</sup>		21.6	4.4	5.8	4.8	16.9	23.3	9.6

\*\* and \*\*\* Significant at .01 and .001 levels, respectively,

NS: non significant at .05.

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, PH: height of plant measured from ground to top of tassel, EH: height from ground to attachment of ear shank, EPR: ratio of height of ear to height of plant, TXT: Grain Texture (1=flint, 5=dent), EA: Ear Aspect (1=conical, 5=cylindrical), GY: visual rating for grain yield (1=excellent grain yield, 5=poor grain yield), EY: grain yield (g ear<sup>-1</sup>).

<sup>§</sup> Fisher's least significant difference, used to compare individual genotypes

<sup>¶</sup>C.V.%: Coefficient of Variation

### *Weslaco 2004*

The mean aflatoxin accumulation for yellow hybrids at Weslaco in 2004 was 368.6 ng g<sup>-1</sup> with a range of 23.68 ng g<sup>-1</sup> to 1963.81 ng g<sup>-1</sup> (Table 3-21). Significant differences were detected for aflatoxin, ear weight, grain texture, plant aspect, root lodging and stalk lodging. No differences were detected for kernel integrity and ear aspect.

Three of the less susceptible hybrids in Weslaco were derived from crosses containing at least one tropical or subtropical parent. Tropical background line (CML415/CML304)-B-2-1-B-B crossed with temperate tester LH195 the least susceptible hybrid. White line (CML269/Tx110)-B-2-B-B-B-B crossed with temperate yellow tester LH195 accumulated the second least aflatoxin. Line (NC300/Tx772)-B-1-B-2-B-B-B crossed with temperate tester LH195 accumulated the third least aflatoxin.

Hybrid (CML415/CML304)-B-2-1-B-B/LH195 had a lower than average grain texture rating (1.9), a lower than average kernel integrity rating (2.1), and average ear aspect rating (2.5) and a higher than average plant aspect rating (2.82). Hybrid (CML269/Tx110)-B-2-B-B-B-B/LH195 had a higher than average grain texture rating (2.84), higher than average kernel integrity rating (2.17), lower than average ear aspect rating (2.2) and higher than average plant aspect rating. Hybrid (NC300/Tx772)-B-1-B-2-B-B-B/LH195 had lower than average grain texture rating (2.21), lower than average kernel integrity rating (2.0), lower than average ear aspect rating (2.19) and lower than average plant aspect rating (1.85).

Table 3-21. Means and statistics of aflatoxin concentration and secondary traits for yellow hybrids at Weslaco in 2004.

Entry	Pedigree	Rank <sup>‡</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	EY g ear <sup>-1</sup>	TXT 1 to 5	KI 1 to 5	EA 1 to 5	PA 1 to 5	RL %	ST %
1	NC300/CML288-B-2-B-B-B/LH195	14	140.5 d-n <sup>\$</sup>	84.57	2.13	1.83	2.80	2.56	5.70	3.56
2	(CML 415/CML304)-B-2-1-B-B/LH195	1	23.7 n	82.66	1.93	2.00	2.50	2.82	15.05	3.50
3	(CML 326/TX772)-B-1-B-B-B/LH195	8	83.2 f-n	75.08	1.32	2.33	2.50	2.29	0.40	0.00
4	(CML 326/TX772)-B-11-B-B-B/LH195	17	187.5 b-m	70.23	1.71	2.17	3.18	1.91	0.10	2.50
5	(CML288/NC300)-B-3-B-B-B/LH195	22	336.5 a-i	90.30	1.99	2.67	2.15	3.37	22.55	3.50
6	(CML288/NC300)-B-9-B1-B-B-B/LH195	18	200.9 b-m	80.32	2.21	1.83	2.77	2.21	1.13	7.33
7	(NC300/TX772)-B-1-B2-B-B-B/LH195	3	63.8 f-n	99.78	2.21	2.00	2.19	1.85	5.50	1.49
8	(TX772/CML326)-B-B5-B-B-B/LH195	5	72.9 f-n	87.27	2.03	2.17	2.45	2.74	15.70	7.03
9	(TX772/CML326)-B-B6-B-B/LH195	4	71.7 f-n	108.75	1.30	2.00	1.95	1.94	0.39	5.27
10	(TX772/CML326)-B-B6-B-B-B/LH195	10	96.1 f-n	69.20	1.67	2.67	3.48	2.83	0.34	12.76
11	(CML269/Tx110)-B-2-B-B-B-B/LH195	2	46.3 i-n	87.54	2.84	2.17	2.20	2.73	5.18	8.75
12	Pop. 69-B-B-B1-6-B-B-B/LH195	7	77.7 f-n	115.15	1.66	1.50	1.79	2.68	21.68	2.46
13	Pop. 69-B-B-B1-8-B-B-B/LH195	9	89.1 f-n	96.72	1.50	1.67	1.81	3.75	8.06	12.89
14	(CML285/B104)-B-4-B-B-B-B/LH210	29	1476.7 a,b	79.99	3.35	2.00	2.69	3.03	1.60	17.93
15	Pop. 69-B-B2-11-B-B-B/LH195	11	104.8 d-n	86.81	1.76	1.67	1.95	2.75	11.15	19.84
16	Pop. 69-B-B-B4-1-B-B-B/LH195	20	246.9 a-k	88.33	1.98	2.50	2.39	2.81	3.86	18.16
17	Pop. 69-B-B-B4-7-B-B-B/LH195	12	115.9 d-n	91.05	1.83	1.83	2.31	2.68	4.61	15.92
18	CML289/Tx772-B-B-B-B-B/LH210	24	532.4 a-g	102.29	2.21	2.00	2.36	2.62	0.47	6.07
19	NC300/CML288-B-1-B-B-B/LH210	27	806.9 a-d	82.55	2.68	2.17	2.83	2.54	3.90	4.11
20	NC300/CML288-B-4-B-B-B/LH210	6	75.7 f-n	96.17	3.17	2.00	2.13	2.31	1.12	0.00
21	NC300/CML288-B-5-B-B-B/LH210	30	1963.8a	94.30	2.29	1.83	2.23	2.20	0.10	0.00
22	Tx770/CML288-B-3-B-B-B/LH210	28	1429.2 a-c	90.73	2.29	1.67	1.88	2.00	0.00	2.32
23	((CML 408/B104)x(CML 411/B104))-2-3-B-B/LH210	23	501.1 a-h	98.18	3.04	2.00	2.36	2.04	0.00	0.65
24	SCR42 x Tx772	21	259.9 a-j	83.96	1.29	2.50	2.96	2.12	2.60	0.34
25	(CML 408/B104)-B-2-1-B-B/LH210	26	775.7 a-e	107.80	3.36	1.67	1.87	2.15	0.00	0.53

Table 3-21 Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF	EY	TXT	KI	EA	PA	RL	ST
			ng g <sup>-1</sup>	g ear <sup>-1</sup>	1 to 5	1 to 5	1 to 5	1 to 5	%	%
26	DKC66-80	16	165.8 d-n	89.86	3.76	2.17	2.65	2.06	1.35	3.08
27	DKC69-70	13	133.6 d-n	84.76	2.71	2.67	2.33	2.09	0.24	0.65
28	P31B13	15	162.9 d-n	75.77	2.18	2.33	3.23	2.23	1.18	0.01
29	P32R25	25	599.4 a-f	80.09	2.36	2.83	3.35	2.75	0.98	1.58
30	LH195 x LH210	19	216.6 b-l	28.3	3.1	3.0	3.3	3.3	2.2	9.7
	Mean		368.6	87.0	2.3	2.1	2.5	2.5	4.6	5.7
	LSD¶		.	2.8	0.6	1.0	1.1	0.9	10.4	12.0
	Sig		**	***	***	NS	NS	**	***	**
	C.V. %#		22.5	6.9	15.2	26.9	26.3	20.7	133.4	122.5

\*\* and \*\*\* Significant at .01 and .001 levels, respectively, NS: non-significant at .05.

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, EY: Ear Yield (average weight of ears after shelling prior to grinding), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), KI: Kernel Integrity (1=no kernels broken to 5=majority of kernels damaged), PA: Plant Aspect (1=desirable to 5=undesirable), RL: Root Lodging (% of plants lodged past 30° of vertical), ST: Stalk Lodging (% of plants lodged below the ear node)

§Mean separations determined using the logarithmic transformation of the data

¶Fisher's least significant difference

#C.V. %: Coefficient of Variation

*Corpus Christi 2004*

The mean aflatoxin accumulation in yellow hybrids at Corpus Christi in 2004 was 31.82 ng g<sup>-1</sup> with a range of 4.30 ng g<sup>-1</sup> to 87.76 ng g<sup>-1</sup> (Table 3-22). Significant differences were detected for all traits measured.

Line (Tx772/CML326)-B-B6-B-B-B crossed with temperate tester LH195 accumulated the least aflatoxin. Line Population 69-B-B-B1-B-B-B crossed with temperate yellow tester LH195 accumulated the second least aflatoxin. Line ((CML408/B104)x(CML411/B104))-2-3-B-B crossed with temperate yellow tester LH210 accumulated the third least aflatoxin.

Hybrid (Tx772/CML326)-B-B6-B-B-B/LH195 had average kernel integrity rating (1.93), lower than average ear aspect rating (2.0), and lower than average grain yield rating (2.0). Hybrid Population 69 Templado Amarillo QPM-B-B-B1-B-B-B/LH195 had lower than average kernel integrity rating (1.56), lower than average ear aspect rating (2.0), lower than average grain texture rating (1.67) and lower than average grain yield rating (2.0). Hybrid ((CML408/B104)x(CML411/B104))-2-3-B-B/LH210 had higher than average kernel integrity rating (2.60), lower than average ear aspect rating (2.33), higher than average grain texture rating (3.17), and lower than average grain yield rating (2.17).

Overall at Corpus Christi, lines on tester LH195 accumulated less aflatoxin than did lines crossed with tester LH210 and that hybrid LH195/LH210 accumulated 48.61 ng g<sup>-1</sup> (Table 3-22).



Table 3-22. Means and statistics for aflatoxin concentration and secondary traits for yellow hybrids at Corpus Christi in 2004.

Entry	Pedigree	Rank <sup>†</sup>	AF <sup>‡</sup> ng g <sup>-1</sup>	KI 1 to 5	EA 1 to 5	TXT 1 to 5	GY 1 to 5
1	NC300/CML288-B-2-B-B-B/LH195	13	12.5 d-o <sup>\$</sup>	1.97	3.17	2.33	3.17
2	(B104/NC300)-B-1-B1-B-B/LH195	21	48.2 a-h	2.65	3.17	2.83	3.17
3	(CML 326/TX772)-B-1-B-B-B-B/LH195	15	21.2 a-n	2.28	2.00	1.67	2.50
4	(CML 326/TX772)-B-11-B-B-B-B/LH195	27	56.7 a-d	2.84	2.83	1.67	2.83
5	(CML288/NC300)-B-3-B-B-B-B/LH195	17	33.9 a-k	2.17	2.00	2.00	1.83
6	(CML288/NC300)-B-9-B1-B-B-B/LH195	14	17.4 b-o	2.28	2.83	2.33	3.00
7	Tx714/CML323-B-2-B-B-B/LH210	12	19.7 a-o	2.16	2.00	2.00	2.00
8	(TX772/CML326)-B-B5-B-B-B/LH195	8	16.6 b-o	1.83	2.00	1.50	1.83
9	(TX772/CML326)-B-B6-B-B/LH195	10	13.1 c-o	2.17	1.83	1.67	1.67
10	(TX772/CML326)-B-B6-B-B-B/LH195	1	4.3 o	1.93	2.00	1.67	2.00
11	((B104/NC300)x(CML 323/Tx601y))-1-2-B-B/LH210	18	39.5 a-j	2.87	3.17	3.00	2.83
12	Pop. 69-B-B-B1-6-B-B-B/LH195	4	9.5 i-o	2.04	2.33	2.17	2.33
13	Pop. 69-B-B-B1-8-B-B-B/LH195	2	7.0 l-o	1.56	2.00	1.67	2.00
14	(CML285/B104)-B-4-B-B-B-B/LH210	16	31.6 a-l	2.93	2.00	3.17	1.83
15	Pop. 69-B-B-B2-11-B-B-B/LH195	6	12.7 d-o	1.42	2.33	1.67	2.17
16	Pop. 69-B-B-B4-1-B-B-B/LH195	9	17.0 b-o	1.46	2.33	1.83	2.17
17	Pop. 69 -B-B-B4-7-B-B-B/LH195	5	11.1 f-o	1.56	1.83	1.67	2.17
18	CML289/Tx772-B-B-B-B-B/LH210	25	58.9 a-c	2.93	3.33	1.50	3.50
19	NC300/CML288-B-1-B-B-B/LH210	20	23.4 a-m	2.11	2.83	2.33	3.00
20	NC300/CML288-B-4-B-B-B/LH210	30	87.8 a	2.90	2.00	3.00	2.00
21	NC300/CML288-B-5-B-B-B/LH210	19	42.7 a-i	2.55	2.00	2.17	2.33
22	Tx770/CML288-B-3-B-B-B/LH210	29	53.7 a-e	3.51	2.67	2.67	2.83
23	((CML 408/B104)x(CML 411/B104))-2-3-B-B/LH210	3	9.9 i-o	2.60	2.33	3.17	2.17
24	SCR42 x Tx772	7	10.3 i-o	1.20	2.17	1.00	2.17
25	((CML 325/B104)x(CML294/B104))-2-3-B-B/LH210	24	49.0 a-g	2.57	3.00	2.67	2.83

Table 3-22. Continued.

Entry	Pedigree	Rank <sup>†</sup>	AF	KI	EA	TXT	GY
			ng g <sup>-1</sup>	1 to 5	1 to 5	1 to 5	1 to 5
26	DKC66-80	11	21.4 a-n	3.55	2.83	3.50	2.17
27	DKC69-70	23	58.4 a-c	2.65	1.50	2.50	1.33
28	P31B13	26	49.7 a-f	2.63	2.33	2.50	1.00
29	P32R25	28	69.2 a ,b	3.56	3.00	2.33	1.50
30	LH195 x LH210	22	48.6 a-g	2.79	1.83	3.50	2.17
	Mean		31.8	2.0	3..1	2.3	3.1
	LSD <sup>¶</sup>		.	0.9	0.9	0.4	0.9
	Sig		**	***	***	***	***
	C.V.% <sup>#</sup>		40.7	24.7	16.7	11.4	18.0

<sup>†</sup>Rank of genotypes by Aflatoxin concentration

<sup>‡</sup>Traits are: AF: antilogarithmic transformation of data, KI: Kernel integrity (1=no kernels broken to 5=majority of kernels damaged), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), TXT: Grain Texture (1=flint to 5=dent), GY: visual rating for grain yield (1=high grain yield to 5=low grain yield).

<sup>§</sup>Mean separations determined using the logarithmic transformation of the data

<sup>¶</sup>Fisher's least significant difference

<sup>#</sup>C.V. %: Coefficient of Variation

### *Across Locations 2004*

Data for twenty three hybrids and three locations were combined for across location analysis and BLUP calculation for aflatoxin and grain texture. Significant differences were detected for aflatoxin and grain texture. The mean aflatoxin accumulation was  $90.0 \text{ ng g}^{-1}$  with a range of  $25.6 \text{ ng g}^{-1}$  to  $269.2 \text{ ng g}^{-1}$  (Table 3-23).

Line (Tx772/CML326)-B-B6-B-B-B crossed with LH195 accumulated the least aflatoxin across locations ( $25.6 \text{ ng g}^{-1}$ ) and had lower than average grain texture rating (1.7) (Table 3-23). Two lines derived from Population 69 were less susceptible to aflatoxin accumulation across locations. Line Pop. 69-B-B-B1-8-B-B-B/LH195 accumulated the second least aflatoxin ( $31.9 \text{ ng g}^{-1}$ ) and had lower than average grain texture rating (1.6) (Table 3-23). Line Pop. 69-B-B-B1-6-B-B-B/LH195 accumulated the third least aflatoxin ( $43.0 \text{ ng g}^{-1}$ ) and had a lower than average grain texture rating (1.8) (Table 3-23).

The BLUPs for aflatoxin were distributed around the mean, with the lowest accumulation being predicted at  $43.4 \text{ ng g}^{-1}$  versus  $25.6 \text{ ng g}^{-1}$  for the mean (Table 3-23). The highest BLUP was  $69.8 \text{ ng g}^{-1}$  versus  $269.2 \text{ ng g}^{-1}$  for the mean. The BLUPs for grain texture vary from the means only slightly, mainly by .1 (Table 3-23).

Table 3-23. Means and statistics for aflatoxin and grain texture for yellow hybrids across three Texas locations in 2004.

Entry	Pedigree	Rank <sup>‡</sup>	AF <sup>†</sup>		TXT	
			Mean	BLUP <sup>§</sup>	Mean	BLUP
			ng g <sup>-1</sup>	ng g <sup>-1</sup>	1 to 5	1 to 5
1	NC300/CML288-B-2-B-B-B/LH195	4	44.9 e-h <sup>¶</sup>	60.2	2.1	2.1
3	(CML 326/TX772)-B-1-B-B-B/LH195	8	66.7 d-h	75.7	1.5	1.5
4	(CML 326/TX772)-B-11-B-B-B-B/LH195	16	119.6 a-d	106.1	1.7	1.7
5	(CML288/NC300)-B-3-B-B-B/LH195	14	102.3 b-f	96.9	2.0	2.0
6	(CML288/NC300)-B-9-B1-B-B-B/LH195	9	75.1 d-g	81.0	2.1	2.1
8	(TX772/CML326)-B-B5-B-B-B/LH195	7	60.1 d-h	71.2	1.7	1.7
9	(TX772/CML326)-B-B6-B-B/LH195	5	57.7 d-h	69.6	1.5	1.5
10	(TX772/CML326)-B-B6-B-B-B/LH195	1	25.6 h	43.4	1.7	1.7
12	Pop. 69-B-B-B1-6-B-B-B/LH195	3	43.0 f-h	58.6	1.8	1.8
13	Pop. 69-B-B-B1-8-B-B-B/LH195	2	31.9 g,h	49.3	1.6	1.6
14	(CML285/B104)-B-4-B-B-B-B/LH210	17	137.7 a-d	115.1	3.1	3.0
15	Pop. 69-B-B-B2-11-B-B-B/LH195	6	58.1 d-h	69.9	1.7	1.7
16	Pop. 69-B-B-B4-1-B-B-B/LH195	12	95.0 c-f	92.9	1.8	1.8
17	Pop. 69-B-B-B4-7-B-B-B/LH195	11	83.4 d-f	86.1	1.6	1.6
18	CML289/Tx772-B-B-B-B-B/LH210	18	140.5 a-d	116.5	1.9	1.9
19	NC300/CML288-B-1-B-B-B/LH210	20	148.7 a-d	120.4	2.4	2.4
22	Tx770/CML288-B-3-B-B-B/LH210	23	269.2 a	169.8	2.3	2.3
23	((CML 408/B104)x(CML 411/B104))-2-3-B-B/LH210	15	114.8 a-e	103.6	3.1	3.1
24	SCR42 x Tx772	10	76.6 d-g	82.0	1.1	1.2
26	DKC66-80	13	100.0 b-f	95.7	3.6	3.5
27	DKC69-70	19	144.2 a-d	118.2	2.4	2.4
28	P31B13	22	248.7 a,b	162.1	2.3	2.3
29	P32R25	21	234.4 a-c	156.7	2.3	2.3
	Mean		90.0	95.7	2.1	2.1
	LSD <sup>#</sup>		.		0.3	
	Gen. Sig.		**		***	
	Env. Sig.		***		*	
	Gen.*Env. Sig.		NS		NS	
	C.V. % <sup>††</sup>		22.9		15.7	

\*, \*\* and \*\*\* Significant at .05 .01 and .001 levels, respectively,

NS: non-significant at .05.

† Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint to 5=dent).

‡ Rank of genotypes by Aflatoxin concentration

§ BLUP: Best Linear Unbiased Predictor

¶ Mean separations determined using the logarithmic transformation of the data

# Fisher's least significant difference

†† C.V. %: Coefficient of Variation

## Conclusions

Multiyear and multilocation evaluations to assess response to aflatoxin of yellow hybrids were conducted from 1999 to 2004. Several experimental hybrids had lower susceptibility to aflatoxin contamination than commercial hybrids in these evaluations. The parental lines involved in these hybrids can be used as new germplasm to develop less susceptible hybrids. Hybrids involving line Tx772 as a parent were across years and locations the less susceptible to aflatoxin contamination. However, even these hybrids showed different and contrasting aflatoxin accumulations across environments. This suggests a heavy environmental component in aflatoxin accumulation.

Hybrids containing tropical and subtropical lines CML285, CML288, CML323, CML325, CML326 and CML338 from CIMMYT with hard kernel texture and long, tight husk cover tended to accumulate less aflatoxin than the commercial checks.

Hybrids containing semi-tropical line Tx601y tended to accumulate less aflatoxin than commercial hybrid checks. Lines derived from Tx772 as a single parent tended to perform well and had less aflatoxin when crossed with LH195. Several of the lines are selections from the same cross, and perform differently for aflatoxin accumulation. Even though ranges of aflatoxin accumulation occurred in lines derived from crosses where Tx772 was at least one parent, overall aflatoxin accumulation was lower than from other single parents of hybrids.

Higher accumulations of aflatoxin were experienced in Mp715 and population MAS gk were noted in 1999. The preceding were released as sources of maize for the

reduction of aflatoxin (Scott and Zummo, 1999, Widstrom and McMillian), but were not effective in reducing aflatoxin in our tests.

While secondary characteristics measured in these trials and as suggested by Barry *et al.* (1986), Betran and Isakeit (2004), Bhatnagar et al. (2003), Odvody (1997) may be part of lowering susceptibility of maize to aflatoxin, those characteristics are not the only factors which affect susceptibility of maize to aflatoxin accumulation. Even with the best physical barriers, environmental conditions may be favorable for *Aspergillus flavus* growth and aflatoxin accumulation. Hybrids with some of the best kernel integrity ratings still accumulated high aflatoxin in particular locations and years. Norton (1997) and Wicklow et al. (1998) have identified  $\beta$ -carotene in maize kernels as a source of chemical resistance. Several of the inbreds utilized in the trials have dark orange kernels, possibly high in  $\beta$ -carotene and may need to be analyzed for that possibility.

Several TAMU experimental lines had ear yields comparable to or exceeding those of the commercial hybrids and had aflatoxin concentrations lower than the commercial checks. Increased yield and improved agronomic qualities, along with resistance to aflatoxin contamination is the goal of our program.

Overall, breeding maize for improved host plant resistance to aflatoxin can be accomplished and has been previously reported in germplasm such as MP313, MP420, MP715, MP717, Tx601y and Tx807 (Scott and Zummo, 1989, Scott and Zummo, 1992, Williams and Windham 1997, Williams and Windham, 2006). The concurrent goal of breeding maize is for resistance to aflatoxin and producing a genotype which has good

agronomic characteristics. Current breeding efforts have succeeded in one of the selections. Agronomic qualities such as maturity, good combining ability and good grain characteristics need to be brought into aflatoxin resistant maize lines to be deployed in farmers' fields.

## **CHAPTER IV**

### **AFLATOXIN EVALUATION OF MAIZE INBREDS**

#### **Objectives**

The objective of this section is to evaluate the progress of the Maize Breeding and Genetics Program's accomplishments of breeding maize for the reduction in susceptibility of aflatoxin accumulation in yellow inbreds in *per se* aflatoxin trials.

#### **Materials and Methods**

##### *Germplasm*

Four years of aflatoxin evaluations of yellow inbreds were conducted at one or two Texas locations (Figure 3.1). Inbreds with diverse origins and genetic backgrounds were evaluated *per se* to estimate their value to reduce aflatoxin and the expression of resistant factors. The inbreds consist of tropical lines from CIMMYT (CML's), subtropical lines from Texas (Tx601y) and North Carolina (NC300), temperate lines from the U.S. Midwest (B104, B97 and FR2128) and locally adapted lines bred and selected in Texas (Tx's), lines derived from Population 69 from CIMMYT and previously reported aflatoxin resistant lines (Mp's and MAS gk). Most of the Tx inbreds were developed from the introgression of exotic germplasm and selected for those traits which may be related to the reduction of aflatoxin accumulation in maize. A complete listing of pedigrees of inbreds can be found in Table 4-1 in the results section.



### *Field Evaluation of Aflatoxin*

An alpha lattice experimental design was used for all trials with four replications. Experimental units consisted of one row plots. Trials were planted in the spring, at or later than optimal planting time. Drought stress on trials was induced by either withholding irrigation at College Station and Weslaco. *Aspergillus flavus* isolate NRRL3357 was used in the inoculations. A conidial suspension containing  $3 \times 10^7$  conidia of *A. flavus* in 3 mL distilled water was injected 6 to 10 d after mid silk by the silk channel inoculation technique (Zummo and Scott, 1989) or by placing colonized kernels in the row between plots (Odyssey et al., 2000). Approximately 1 kg of colonized maize kernels was applied per 200 feet of row length when using the colonized kernel method of inoculation.

### *Aflatoxin Quantification*

All of the plants in an experimental unit were harvested in trials inoculated using the colonized kernel method while only inoculated plants were harvested in trials inoculated using the silk channel method. Samples were shelled with a maize sheller and the grain was ground using a Romer Mill (Romer Labs, Union, MO). Quantification of aflatoxin was conducted in 50 g subsamples from each plot with monoclonal antibody affinity columns and fluorescence determination by the Vicam Aflatest (Watertown, MA).

### *Statistical Analysis*

Single location data were analyzed as Randomized Complete Block Design and Alpha Lattice Incomplete Block, using SAS Proc GLM and Proc Mixed and using

REMLTool. Aflatoxin concentration was log transformed (base 10) to standardize variances and reported as a geometric mean (antilogarithm). Aflatoxin concentration was expressed in nanograms per gram ( $\text{ng g}^{-1}$ ). Means obtained with the most efficient analysis (i.e., having the lowest error) were reported.

## Results

The results for inoculated inbred *per se* trials across five environments and four years are listed in Table 4-1. The mean aflatoxin accumulation in year 2000 was 535.9  $\text{ng g}^{-1}$  with a range of 25.7  $\text{ng g}^{-1}$  to 1258.9  $\text{ng g}^{-1}$ . Significant differences were detected for aflatoxin accumulation. Two inbred lines released for lowered susceptibility to aflatoxin accumulation were two lines less susceptible to aflatoxin accumulations in 2000 (Llorente et al., 2004 and Williams and Windham, 2001). Inbred Tx772 with its hard flinty orange kernel accumulated less aflatoxin, had good husk cover, hard grain texture rating and poor grain yield rating. Inbred Mp715 was the second less aflatoxin, had good husk cover, hard grain texture and an average grain yield rating. However Mp715 lacks favorable agronomic characteristics to be directly used in commercial hybrids. Line Tx772 accumulated significantly less aflatoxin than lines Mp715 and Mp420, and population MAS gk.

Aflatoxin accumulation in the 2001 yellow inbred trial in Weslaco had a mean of 1231.02  $\text{ng g}^{-1}$  with a range of 407.38  $\text{ng g}^{-1}$  to 2754.23  $\text{ng g}^{-1}$ . Significant differences for aflatoxin accumulation were detected. Two tropical/subtropical lines, one with previous history of aflatoxin resistance, were among the three lines less susceptible to aflatoxin accumulations in 2001. Tropical line CML289 accumulated less aflatoxin, had

good husk cover, an average grain texture rating, higher amounts of insect damage and poor grain yield. Line Tx601y accumulated second least aflatoxin, had excellent husk coverage, similar grain texture to CML289, but had very poor grain yield in the trial. Inbred Tx732 accumulated third least aflatoxin, had average husk coverage, more dent kernel texture but differed from the other two inbreds by having a higher yield rating.

Two locations of the yellow inbred line trial were planted in 2002, one at College Station and the other at Weslaco. College Station, utilized the colonized kernel method of inoculating. This method of inoculation, along with high levels of rain and furrow irrigation, did not discriminate well among genotypes at College Station and produced a wide range of aflatoxin accumulations and rather high coefficient of variation (74%) (Table 4-1). The mean aflatoxin accumulation at College Station was  $56.16 \text{ ng g}^{-1}$  with a range of  $1.0 \text{ ng g}^{-1}$  to  $543.9 \text{ ng g}^{-1}$  (Table 4-1). The four lowest aflatoxin accumulations were separated by only  $0.7 \text{ ng g}^{-1}$ .

Experimental conditions at Weslaco in 2002 were more conducive for aflatoxin accumulations than those in College Station during the same year. The mean aflatoxin accumulation was  $324.9 \text{ ng g}^{-1}$  with a range of  $5.59 \text{ ng g}^{-1}$  to  $1490.22 \text{ ng g}^{-1}$  (Table 4-1). Two southern adapted inbred lines and a tropical inbred line compose the three least susceptible inbred lines in Weslaco. Inbred line Tx732 accumulated the least aflatoxin ( $5.59 \text{ ng g}^{-1}$ ), exhibited a medium grain texture and had higher incidence of insect damage. Inbred line Tx714 accumulated second least aflatoxin  $13.27 \text{ ng g}^{-1}$ , had a harder kernel texture than Tx732 and had a high incidence of insect damage. Tropical line CML285 accumulated the third least aflatoxin ( $31.86 \text{ ng g}^{-1}$ ), exhibited a hard flinty kernel type and an elevated incidence of insect damage.

The mean aflatoxin accumulation at Weslaco in 2003 was  $353.73 \text{ ng g}^{-1}$  with a range of  $9.63 \text{ ng g}^{-1}$  to  $2205.62 \text{ ng g}^{-1}$  (Table 4-1). Line Tx772 accumulated less aflatoxin ( $9.63 \text{ ng g}^{-1}$ ), exhibited a hard kernel texture and good kernel integrity. Inbred lines derived from Population 69 showed impressive resistance to aflatoxin accumulation. Three of the lines, Pop. 69-B-B-B4-7-B-B, Pop. 69-B-B-B2-2-B-B and Pop. 69-B-B-B3-6-B-B (in order of aflatoxin accumulation), were statistically similar to the lowest accumulating inbred Tx772. All six of the Pop.69 lines were statistically similar; all exhibited flinty kernel type and had excellent kernel integrity.

Table 4-1. Means and statistics for aflatoxin concentrations for yellow inbred *per se* trials at two environments and four years.

Genotype	2000CS <sup>†</sup>	2001WE	2002 CS	2002 WE	2003WE
	<u>ng g<sup>-1</sup></u>	<u>ng g<sup>-1</sup></u>	<u>ng g<sup>-1</sup></u>	<u>ng g<sup>-1</sup></u>	<u>ng g<sup>-1</sup></u>
Mp715	112.2 c <sup>‡</sup>				
MP420	707.9 a,b				
MAS gk	1000.0 a				
Tx714		1757.9 a-c	34.7 b-d	13.3e,f	820.7 a,b
Tx732		441.6 g-h	28.4 b-d	5.6 f	584.5 a-c
Tx601y	234.4 b,c	429.0 g-h	1.0 e	..	..
Tx760			74.6 a-c	87.4 b-e	144.3 c,d
Tx770		540.1 f-h	23.0 b-d	1490.2 a	
Tx772	25.7 d	2317.4 a-b	1.7 e	254.0 a-d	9.6 g
B104		1122.0 b-f	8.5 b-e	35.4 d-f	368.2 b,c
B97		2053.5 a-c	543.9 a	611.3 a,b	2205.6 a
NC300		1209.2 b-f	1.3 e	196.6 a-d	37.5 d-g
FR2128	501.2 a,b	1612.5 a-d	8.7 b-e	66.3 c-e	364.1 b,c
CML285	446.7 a,b	2754.2 a	1.7 e	31.9 d-f	17.3 e-g
CML288		966.1 c-g	1.8 e	107.1 b-e	477.7 b,c
CML289		407.4 h			
CML294		676.1 e-h			
CML323		724.4 d-h	84.3 a,b	265.2 a-d	628.5 a-c
CML 325			24.7 b-d	1189.9 a	936.5 a,b
CML 326	1258.9 a				
CML338		1453.8 a-e	4.1 d,e	518.8 a-c	74.2 d,e
Pop. 69 -B-B-B2-2-B-B					15.4 f,g
Pop. 69 -B-B-B3-5-B-B					53.6 d-f
Pop. 69 -B-B-B3-6-B-B					21.9 e-g
Pop. 69 -B-B-B4-7-B-B					9.6 g
Pop. 69 -B-B-B4-11-B-B					47.2 d-f
Pop. 69 -B-B-B5-7-B-B					48.8 d-f
Mean	535.9	1231.0	56.2	324.9	353.7
LSD		.	.	.	.
Sig	***	***	**	***	***
C.V. % <sup>§</sup>	17.0	8.31	74.0	33.3	23.5

\*\* and \*\*\* Significant at the .01 and .001 Levels, respectively

<sup>†</sup> Trials were grown at: CS: College Station, TX and WE: Weslaco, TX<sup>‡</sup> Mean separations determined using the logarithmic transformation of the data. Entries with the same letter are not significantly different at the .05 level.<sup>§</sup> C.V. %: Coefficient of Variation

## Discussion and Conclusions

Aflatoxin accumulations in inbreds *per se* appear to be variable from year to year. Significant differences were observed for aflatoxin accumulations in maize inbreds *per se*. These differences may be dependent on several factors including grain texture, kernel integrity, susceptibility to insect damage, husk tightness and husk coverage.

With the exception of year 2001, inbred line Tx772 exhibited more resistance to aflatoxin accumulations than other lines, which have shown previous resistance to aflatoxin accumulations.

Inbred lines derived from Population 69 show promising results for aflatoxin accumulations. These lines typically have hard flinty endosperm and have long tight husk cover. Inbred lines derived from Population 69 are higher in lysine, which is limiting in the majority of maize planted. Further testing of these lines will be necessary to determine agronomic qualities for potential use in commercial hybrids.

Inbred line Tx601y in hybrid combinations has shown lowered susceptibility to aflatoxin accumulations in previous evaluations. Inbred line Tx601y has shown over the three years of testing for aflatoxin accumulation varied response. Several of the CIMMYT inbred lines had lower accumulations within individual years, but lacked having consistent low accumulations over years. In 2001 several of the CIMMYT lines (CML289, CML294 and CML323) had reduced aflatoxin being statistically ( $P > .05$ ) similar and ranked in the top for aflatoxin resistance.

Evaluating maize lines *per se* was useful as a preliminary screening before evaluating the lines in hybrid combinations. Evaluating maize inbreds *per se*, due to potential and recurrent variability of lines across years may help identify those lines with resistance which will not be missed under certain environmental conditions and which could be masked in hybrid combinations due to relevant gene action within the line.

## **CHAPTER V**

### **EVALUATION OF *ASPERGILLUS FLAVUS***

### **ISOLATES AND MAIZE GENOTYPES**

#### **Introduction**

Variability of *Aspergillus flavus* isolates in nature is a factor which needs to be taken into account in a screening program for *A. flavus* and aflatoxin resistance. The capability to produce aflatoxins and the amount of aflatoxin produced vary across isolates and environments (Cotty, 1997; Ramaswamy, 2002). The most common method of inoculating screening genotypes with *A. flavus* in breeding programs involves one single isolate, chosen for its capacity to produce aflatoxin (Windham and Williams, 2002; Scott and Zummo, 1988; Campbell and White, 1995; Barry et al., 1992; Lillehoj et al., 1975; Lillehoj et al., 1978).

Isolates used in breeding programs are selected on their ability to produce B1/B2 and G1/G2 aflatoxin. Naidoo et al. (2002) and Campbell and White (1995) used multiple isolates to ensure toxigenicity “in case any one isolate suddenly became non-toxigenic after lab culture” (Naidoo et al., 2002). However, the question arises how different isolates will affect different maize genotypes. In other words, does interaction occur between isolates producing aflatoxin and different genetic back grounds or maize? The objectives of this study were: (1) to determine if there is interaction between isolates of *A. flavus* and maize genotypes under field inoculation; and (2) estimate the aflatoxin producing capacity of the different isolate and the response of maize genotypes.



## **Materials and Methods**

Two field experiments, one with maize inbreds and the other with maize hybrids, were conducted in five environments at three locations (Weslaco, Corpus Christi and College Station, TX) over two years (2004 and 2005).

Inbreds and hybrids were selected for maturity and previous response to aflatoxin under inoculation. The hybrid trial included four commercial and four TAMU experimental hybrids. The commercial hybrids were Dekalb Brand DKC69-70, Asgrow RX949W, Pioneer Brand P31B13, and Crow's Hybrids SR470. The TAMU experimental hybrids were Population 69 Templado Amarillo QPM-B-B-B4-11-B-B-B/LH195, CML161/LH195, Tx114/Tx110 and CML161/CML172. The inbred trial included three white inbreds (Tx114, CML176 and CML269) and two QPM inbreds (Tx804 and CML161). Inbreds from the International Maize and Wheat Improvement Center (CIMMYT) CML176, CML269 and CML161 have shown reduced aflatoxin concentration in past evaluations while inbreds Tx114 and Tx804 have been highly susceptible to aflatoxin.

The hybrid trial was planted at College Station, Weslaco and Corpus Christi in 2004 and at College Station and Weslaco in 2005. The inbred trial was planted at College Station and Weslaco both years. An alpha-lattice field experimental design was used in the hybrid trial, and a randomized complete block design in the inbred trial, both with four reps.

Hybrids and inbreds were inoculated using the non-injuring silk channel inoculation method (Zummo and Scott, 1989) using isolates L1, F1, I5 and NRRL3357 (AF3357). Isolates L1, F1 and I5 were selected from a set of *Aspergillus flavus* isolates collected in a maize field in San Patricio County, Texas (Ramaswamy, 2002) for their ability to produce B1, B2/G1, and G2 aflatoxin. Isolate NRRL3357 has previously shown high levels of aflatoxin production and is currently used by many programs for screening for aflatoxin susceptibility. All isolates were prepared for inoculation using the same protocol. Isolates were inoculated in plants within the same row of maize. Plants inoculated with the same isolate were tagged with colored tape of the same color, which was different among isolates. Three to five ears were inoculated per plot per isolate, depending on final plant population in the field. Plots were hand harvested, keeping ear samples inoculated with the same isolate separated and identified by the colored tape used at inoculation. Plot ear samples were shelled and bulked by isolate. Bulk kernel samples per plot and isolate were ground with a Romer Mill (Romer Labs, Union, MO) and mixed. A 50 g sub sample from the mixed ground was taken and used for quantification of aflatoxin with Vicam Aflatest<sup>®</sup> (VICAM, Watertown, MA).

#### *Statistical Analysis*

Single and across location analysis were conducted using SAS Proc GLM. Aflatoxin concentrations in  $\text{ng g}^{-1}$  were transformed using logarithm (base 10) transformation to standardize variances. Results were reported as antilogarithm values of transformed adjusted means. .

Additive Main Effects and Multiplicative Interaction (AMMI) analysis of aflatoxin in inbreds and hybrids at different environments was carried out to assess the relationship among genotypes and environments using Biplot v1.1 (Dr. E.P. Smith, Virginia Tech; <http://www.stat.vt.edu/facstaff/epsmith.html>).

## Results

### 2004 Hybrid Trial

Considering the variability that occurs when testing for aflatoxin contamination, reps were only significant in the College Station trial and were not significant at Weslaco and Corpus Christi ( $P > .05$ ) (Table 5-1). Significant differences for aflatoxin concentration were detected among maize genotypes and among *A. flavus* isolates at all three locations (Table 5-1). No significant differences were detected at any of the locations for genotype by isolate interaction ( $P < .05$ ) (Table 5-1).

Table 5-1. Analysis of variance for aflatoxin concentration of maize hybrids inoculated with *A. flavus* isolates at three locations in 2004.

Source	DF	Environments		
		CS <sup>†</sup>	WE	CC
		MS	MS	MS
Reps	3	3.49***	0.01	0.12
Genotype	7	1.35***	1.01***	2.62***
Isolate	3	1.13*	1.18**	5.09***
Genotype*Isolate	21	0.46	0.17	0.10
Error	93	0.32	0.22	0.23

\*, \*\*, \*\*\* Significant at .05, .01 and .001 levels, respectively

<sup>†</sup>Locations are CS=College Station, WE=Weslaco and CC=Corpus Christi

DF: degrees of freedom, MSE: Mean Square Error

Isolate I5 produced more aflatoxin at College Station ( $196.42 \text{ ng g}^{-1}$ ) and Weslaco ( $552.71 \text{ ng g}^{-1}$ ) than the other isolates (Figure 5-1). Isolate F1 produced more aflatoxin at Corpus Christi ( $227.22 \text{ ng g}^{-1}$ ) than the other isolates (Figure 5-1).

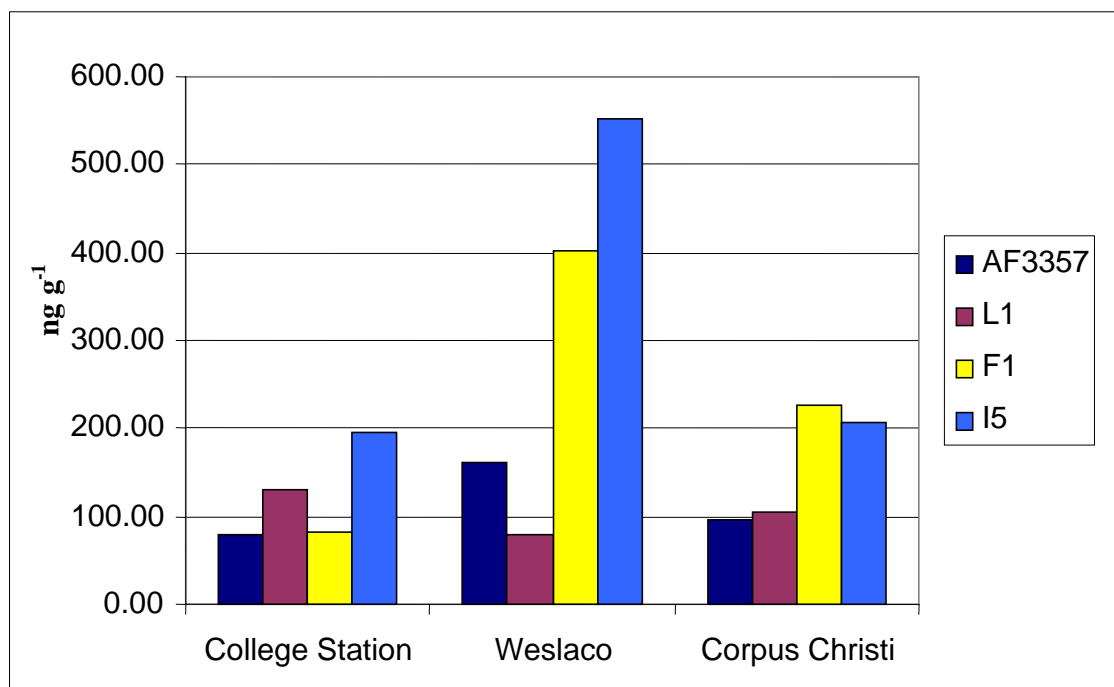


Figure 5-1. Means for aflatoxin concentrations of *A. flavus* isolates in maize hybrids evaluated at three locations in 2004.

Isolate AF3357 produced the most aflatoxin in hybrids Tx114/Tx110 and SR470 at College Station ( $651.63 \text{ ng g}^{-1}$  and  $666.42 \text{ ng g}^{-1}$ , respectively) (Figure 5-2).

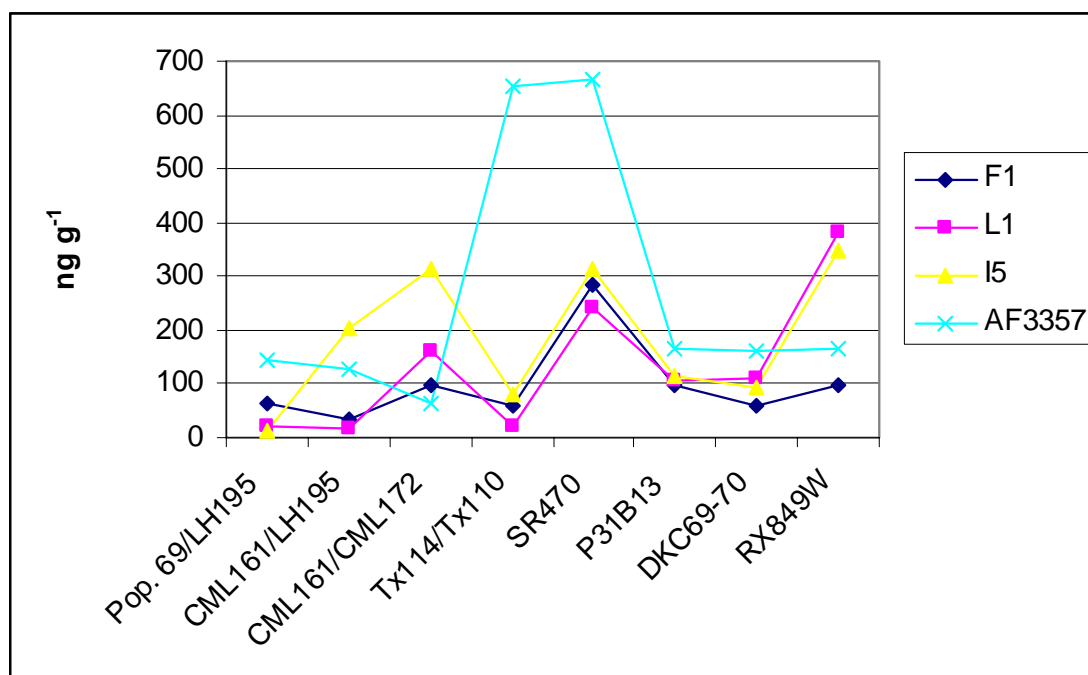


Figure 5-2. Aflatoxin accumulation per *A. flavus* isolate in hybrids at College Station in 2004

Isolates I5 and F1 produced the most aflatoxin in hybrid SR470 at Weslaco (697.83 ng g<sup>-1</sup> and 681.55 ng g<sup>-1</sup>, respectively) (Figure 5-3).

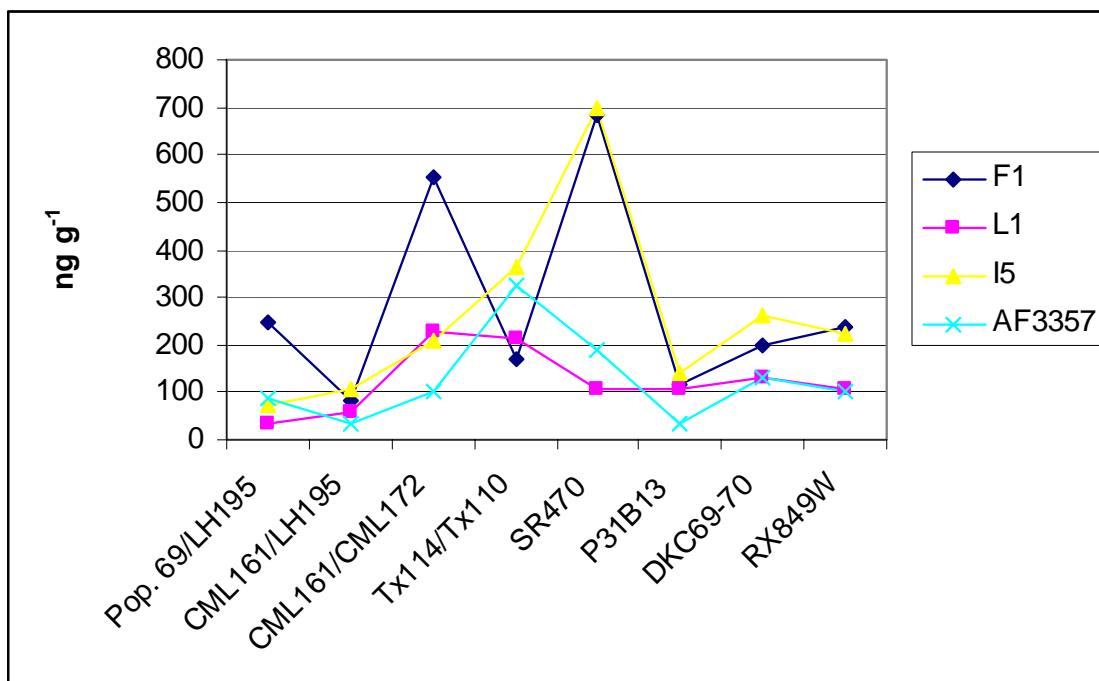


Figure 5-3. Aflatoxin accumulation per *A. flavus* isolate in hybrids at Weslaco in 2004

Isolates F1, I5 and AF3357 produced the most aflatoxin in hybrid CML161/CML172 at Corpus Christi in 2004 (2030.02 ng g<sup>-1</sup>, 1669.17 ng g<sup>-1</sup> and 1621.81 ng g<sup>-1</sup>, respectively) (Figure 5-4). The least overall aflatoxin production from all isolates was for hybrid Pop.69/LH195 (Figure 5-4).

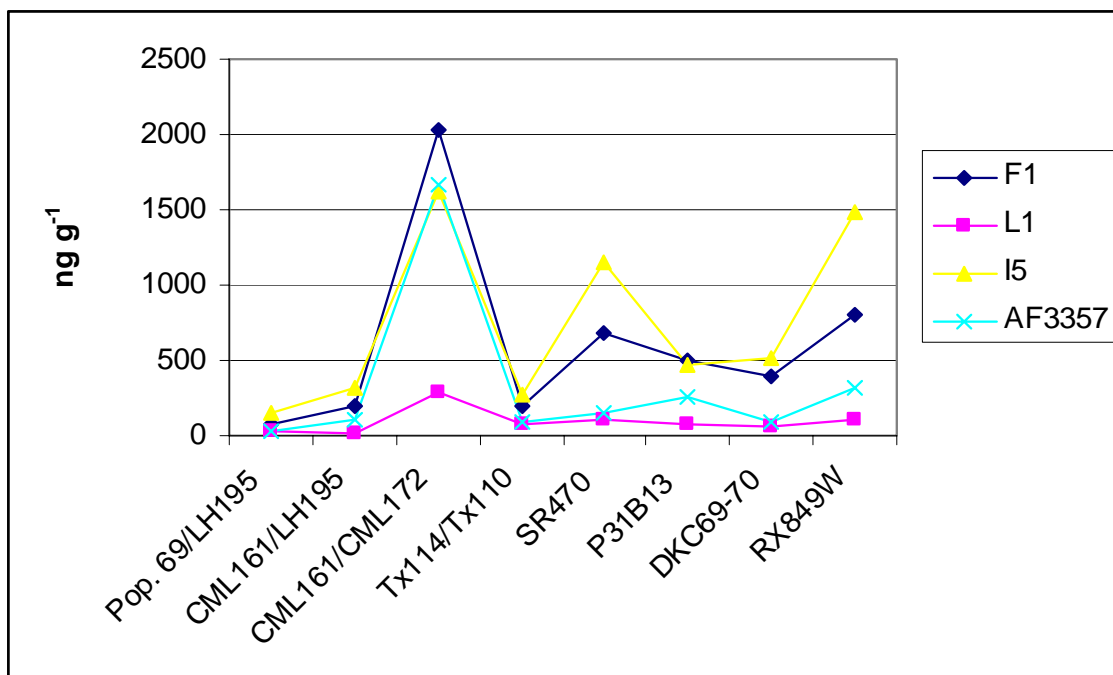


Figure 5-4. Aflatoxin accumulation per *A. flavus* isolate in hybrids at Corpus Christi in 2004

#### *Hybrids Across Locations in 2004*

Significant differences were detected for environment, rep(environment), genotype, isolate, environment by genotype interaction, and environment by isolate interaction sources of variation were detected for across locations analysis in 2004 (Table 5-2). No significant differences were detected for genotype by isolate interaction and environment by genotype by isolate interaction for the hybrid trial across environments in 2004.

Table 5-2. Analysis of variance for aflatoxin concentration of maize hybrids inoculated with *A. flavus* isolates across locations in 2004.

Source of variation	DF	MS
Env <sup>†</sup>	2	3.176***
Rep (Env)	9	1.205***
G	7	3.535***
I	3	4.276***
G*I	21	0.263
Env*G	14	0.666**
Env*I	6	1.320***
Env*G*I <sup>†</sup>	42	0.233
Error	278	0.263

\*\* and \*\*\* Significant at .01 and .001 levels respectively

DF: degrees of freedom. MS: Mean square

<sup>†</sup> Sources of Variation in ANOVA: Env=environment, rep(Env)=Reps within environments, G=genotypes, I=isolates, G\*I=genotype by isolate interaction, Env\*G=environment by genotype interaction, Env\*I=environment by isolate interaction, Env\*G\*I=environment by genotype by isolate interaction.

### 2005 Hybrid Trial

Replications were significant ( $P < .05$ ) at College Station, but they were not significant at Weslaco (Table 5-3). Significant differences ( $P < .05$ ) were detected among hybrid genotypes and among isolates (Table 5-3). No significant differences ( $P < .05$ ) were detected for genotype by isolate interaction at either location (Table 5-3).

Table 5-3. Analysis of variance for aflatoxin concentration of maize hybrids inoculated with *A. flavus* isolates at College Station and Weslaco, TX in 2005.

Source of Variation	DF	CS <sup>†</sup>	We
		MS	MS
Replications	3	0.487*	0.115
Genotypes	7	2.632***	2.873***
Isolates	3	1.192***	1.583***
Genotypes*Isolates	21	0.114	0.123
Error	86	0.164	0.132

\* and \*\*\* Significant at .05 and .001 levels, respectively

<sup>†</sup> Location s are CS=College Station and WE=Weslaco.



Isolate F1 produced more aflatoxin in both College Station (549.15 ng g<sup>-1</sup>) and Weslaco (248.67 ng g<sup>-1</sup>) (Figure 5.5). Isolate I5 produced slightly less aflatoxin in Weslaco (233.19 ng g<sup>-1</sup>) than did isolate F1 (248.67 ng g<sup>-1</sup>)(Figure 5-5).

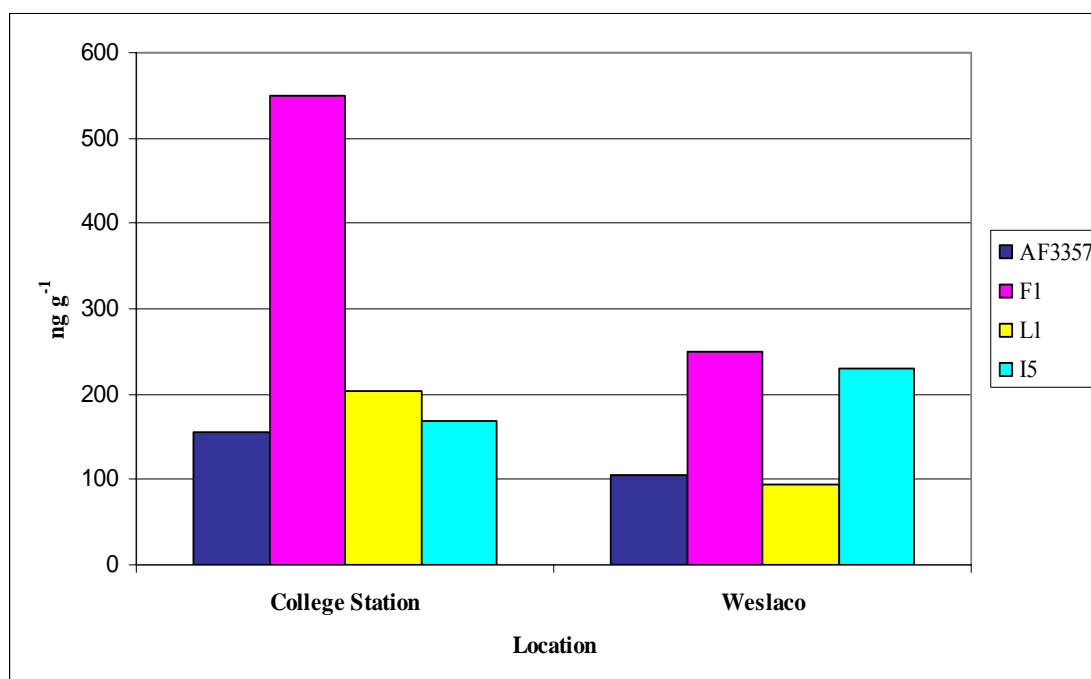


Figure 5-5. Means of aflatoxin concentration per *A. flavus* isolate in hybrid trial at two locations in 2005

All isolates produced the most aflatoxin in hybrid SR470 at College Station in 2005. At this location and year, SR470 is the most susceptible hybrid. All isolates produced the least aflatoxin in hybrid CML161/LH195 at College Station in 2005 (Figure 5-6).

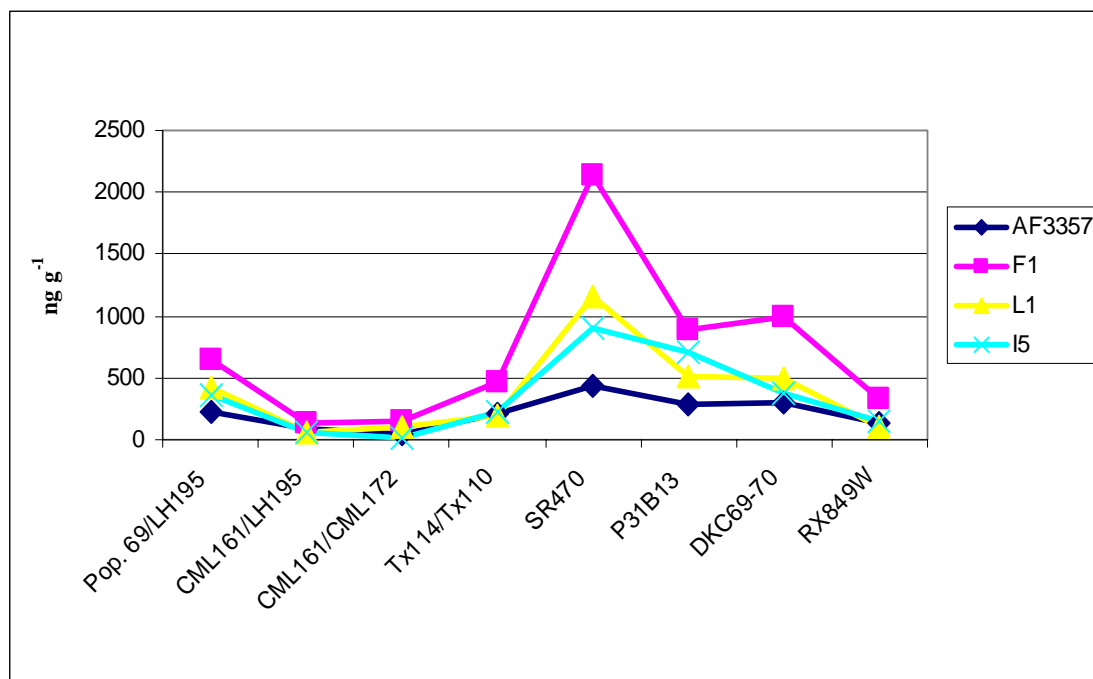


Figure 5-6. Aflatoxin concentration per *A. flavus* isolate in hybrids at College Station in 2005.

Isolates F1 and I5 produced the most aflatoxin in hybrid SR470 at Weslaco (2077.30 ng g<sup>-1</sup> and 1717.91 ng g<sup>-1</sup>, respectively) (Figure 5-7).

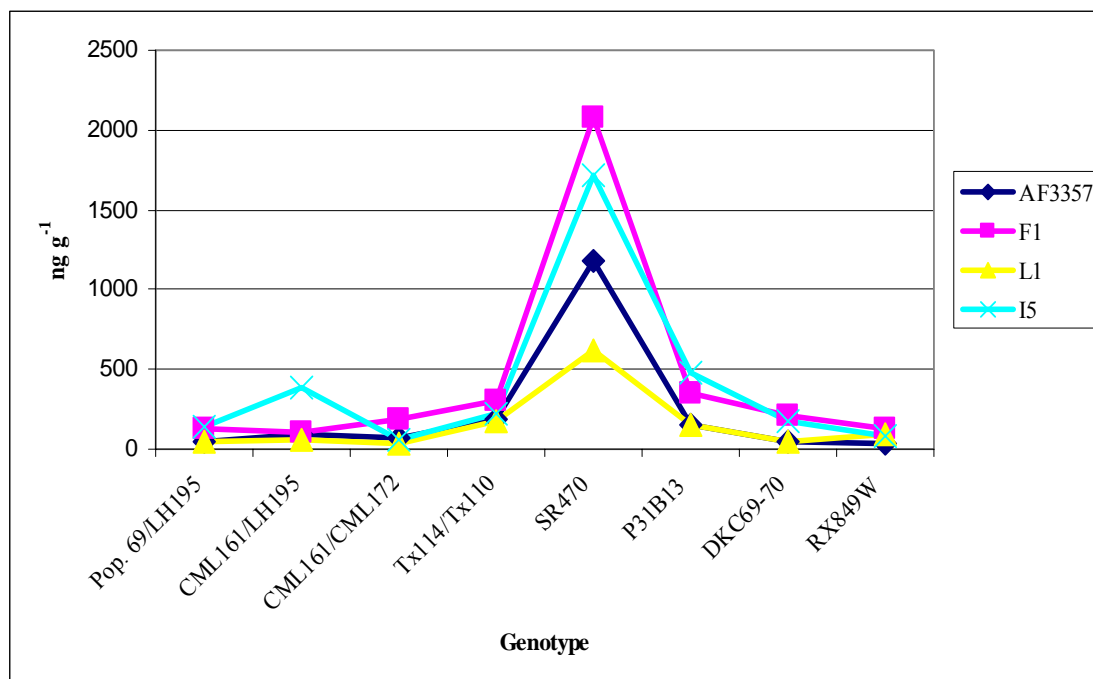


Figure 5-7. Aflatoxin accumulation per *A. flavus* isolate in hybrids at Weslaco during 2005.

#### *Hybrids Across Locations 2005*

Analysis of variance across locations detected significance differences ( $P < .05$ ) for environment, genotype and isolate sources of variation (Table 5-4). No significant differences were observed for genotype by isolate interaction. Significant differences ( $P < .05$ ) were detected for environment by genotype interaction and environment by isolate interaction (Table 5-4).

Table 5-4. Analysis of variance for aflatoxin concentration of maize hybrids inoculated with *A.flavus* isolates at three locations in 2005.

Source <sup>†</sup>	DF <sup>‡</sup>	MS
Env	1	2.037***
Rep (Env)	6	0.397*
G	7	4.571***
I	3	2.786***
G*I	21	0.128
Env*G	7	0.807***
Env*I	3	0.742**
Env*G*I	21	0.129
Error	182	0.155

\*\* and \*\*\* Significant at .01 and .001 levels respectively

† Sources of Variation in ANOVA: Env=environment, rep(Env)=Reps within environments, G=genotypes, I=isolates, G\*I=genotype by isolate interaction, Env\*G=environment by genotype interaction, Env\*I=environment by isolate interaction, Env\*G\*I=environment by genotype by isolate interaction.

‡ DF: degrees of freedom. MSE: Mean square

Significant differences were detected for a comparison of NRRL3357 and the other isolates (Table 5-5). Isolate NRRL3357 showed significant differences with all individual isolates except L1 (Table 5-5).

Table 5-5. Contrasts of *A. flavus* isolate aflatoxin production in hybrids across locations in 2005

Source	DF <sup>†</sup>	MS
NRRL3357 vs. Other	1	2.66***
NRRL3357 vs. F1	1	6.019***
NRRL3357 vs. L1	1	0.019
NRRL3357 vs. I5	1	1.045**

\*\* and \*\*\* Significant at .01 and .001, respectively

† DF: Degrees of Freedom and MS: Mean squares

### *Hybrids Across Locations and Years*

Significant differences for aflatoxin concentration across years 2004 and 2005 were detected for all sources of variation except genotype by isolate interaction and environment by genotype by isolate interaction (Table 5-6). Non statistical interaction between the genotypes and isolates across years reinforces the results observed in individual year analyses.

Table 5-6. Analysis of variance for aflatoxin concentration of maize hybrids inoculated with *A. flavus* isolates across years 2004 and 2005.

Source <sup>†</sup>	DF <sup>‡</sup>	MS
Env	4	3.054***
Rep(Env)	15	0.877***
Genotype	7	4.434***
Isolate	3	5.829***
G*I	21	0.274
Env*G	28	1.515***
Env*I	12	1.087***
Env*G*I	84	0.193
Error	460	0.240

\*\*\* Significant at .001 Level

† DF: Degrees of freedom, MS: Mean Square

\*\* and \*\*\* Significant at .01 and .001 levels respectively

† Sources of Variation in ANOVA: Env=environment, rep(Env)=Reps within environments, G=genotypes, I=isolates, G\*I=genotype by isolate interaction, Env\*G=environment by genotype interaction, Env\*I=environment by isolate interaction, Env\*G\*I=environment by genotype by isolate interaction.

‡DF: degrees of freedom. MS: Mean square

Across locations and years, isolates F1 and I5 produced more aflatoxin than did isolates NRRL3357 and L1 (Figure 5-8). Across locations and years, isolates F1 and I5 produced similar concentrations of aflatoxin and isolates L1 and NRRL3357 produced similar concentrations of aflatoxin.

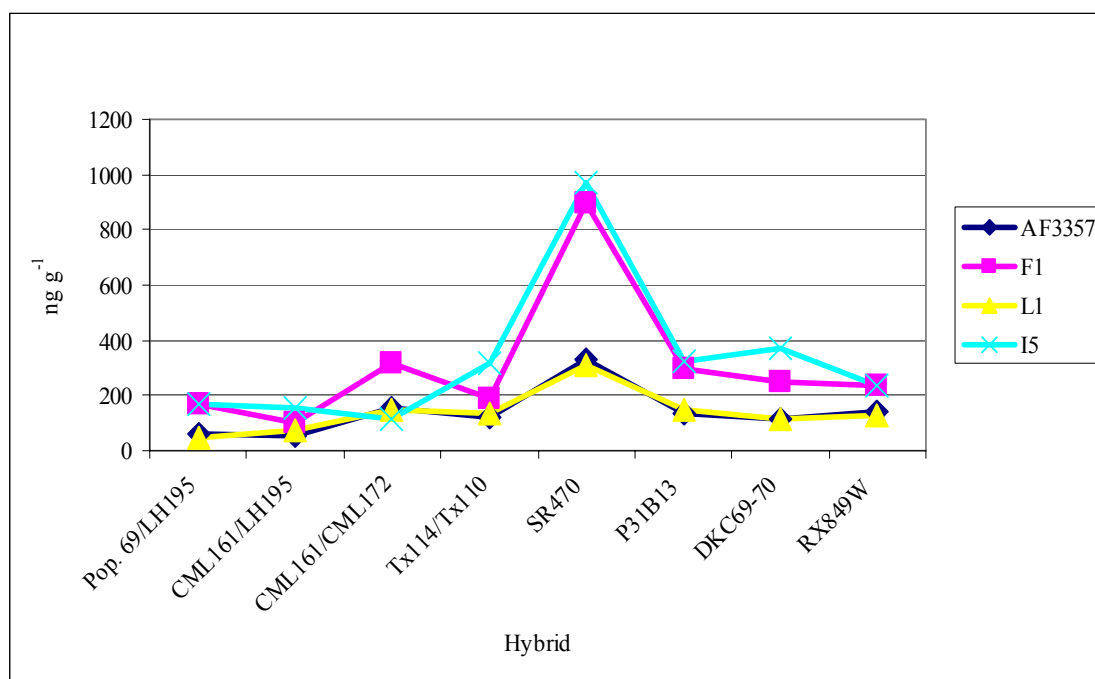


Figure 5-8. Aflatoxin accumulation per *A. flavus* isolate in hybrids across years 2004 and 2005.

Principal component analysis of the interaction between aflatoxin of the different isolates and environments shows different response of isolates I5 and F1, having vectors with wide angle. At contrary, isolates NRRL3357 and L1 response similarly as indicated by the small angle between their vectors (Figure 5-9).

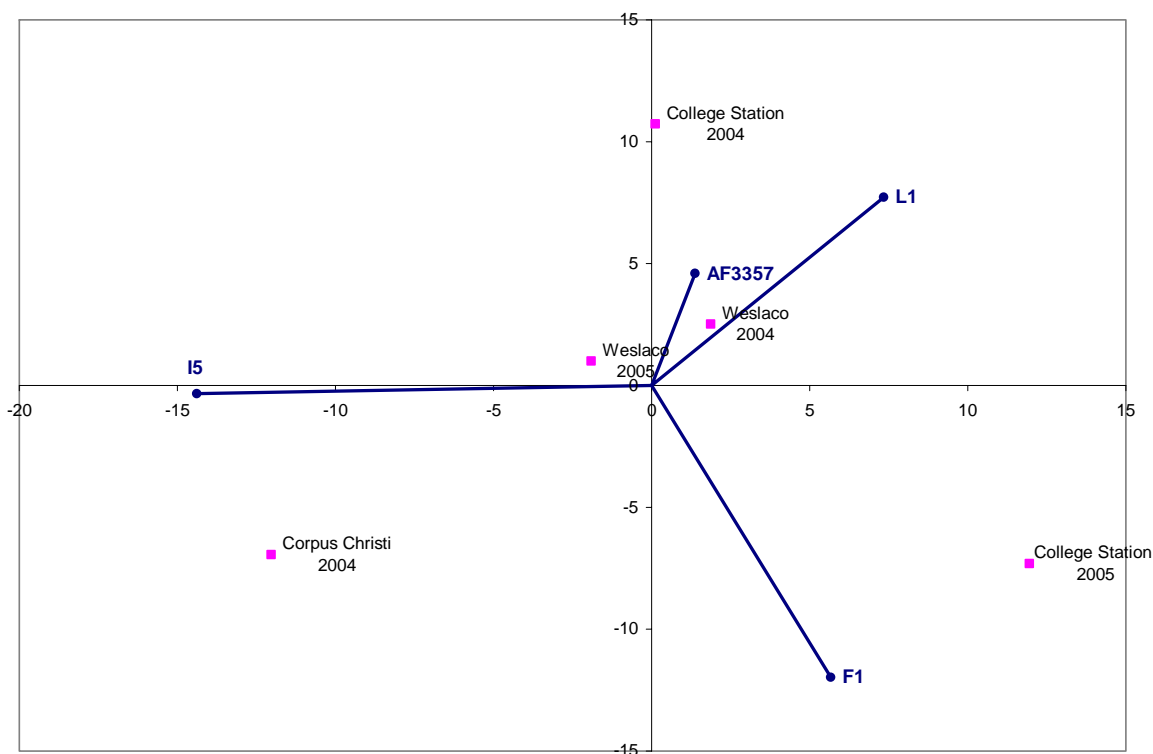


Figure 5-9. Principal component analysis biplot of aflatoxin accumulation by *A. flavus* isolate and locations across years

#### *Inbred Trial 2004*

The results observed in the inbred trial in 2004 were different from those in the hybrid trial. Significant differences ( $P < .05$ ) for aflatoxin accumulation were detected for replications at College Station, but not at Weslaco (Table 5-7). Genotype was significant at both locations, however; isolate was found significant ( $P < .05$ ) at Weslaco but not significant ( $P < .05$ ) at College Station (Table 5-7). No genotype by isolate interaction was detected for inbreds in year 2004 (Table 5-7).

Table 5-7. Analysis of variance for aflatoxin concentration of maize inbreds inoculated with *A. flavus* isolates at two locations in 2004

Source	DF	CS <sup>†</sup>	WE
		MS	MS
Rep	3	0.69*	0.27
Genotype	4	2.05***	4.03***
Isolate	3	0.62	0.62*
Genotype*Isolate	12	0.32	0.20
Error	57	0.24	0.21

\*, \*\*, \*\*\* Significant at .05, .01 and .001 levels, respectively

<sup>†</sup> Locations are CS=College Station and WE=Weslaco

Isolate I5 produced more aflatoxin at College Station (1042.32 ng g<sup>-1</sup>) than did the other isolates (Table 5-8). Isolate F1 was the highest aflatoxin producing isolate at Weslaco (340.68 ng g<sup>-1</sup>) (Table 5-8).

Table 5-8. Mean aflatoxin concentration for each isolate in inbreds at two locations in 2004

Location <sup>†</sup>	F1	L1	I5	AF3357
CS	522.40	408.79	1042.32	518.80
WE	340.68	140.80	329.61	225.19

<sup>†</sup>Locations are CS=College Station and WE=Weslaco

Isolates AF3357 and I5 produced the most aflatoxin in line Tx804 (2213.1 ng g<sup>-1</sup> and 2041.74 ng g<sup>-1</sup>, respectively) (Figure 5-10).



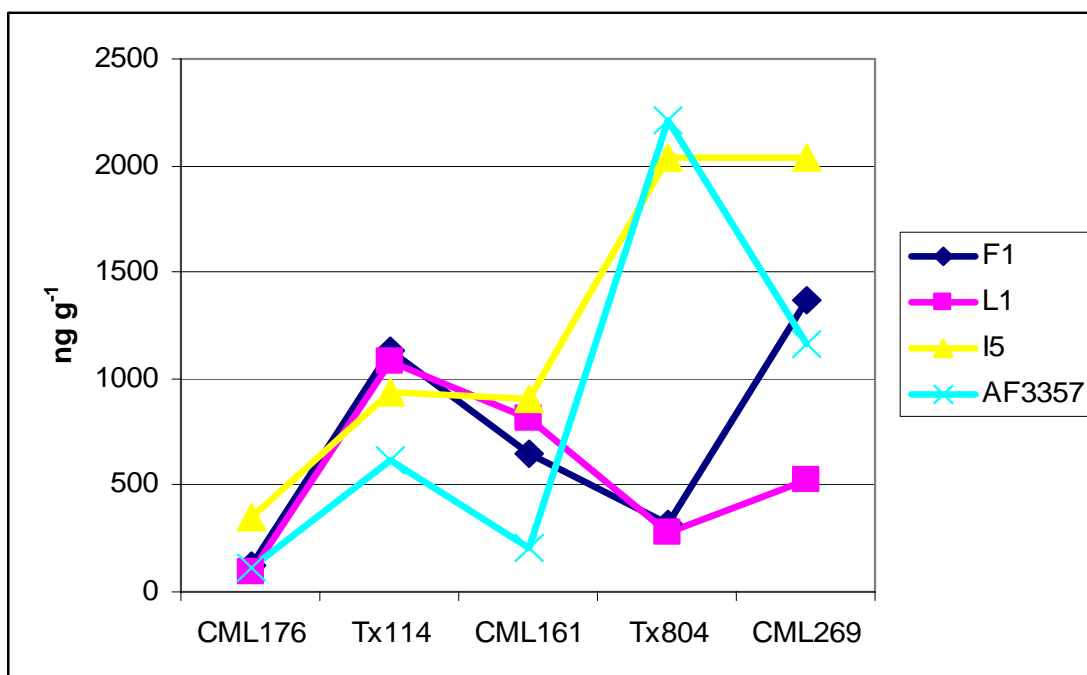


Figure 5-10. Aflatoxin accumulation per *A. flavus* isolate in inbreds at College Station in 2004

Line Tx804 produced the most aflatoxin with three of the isolates in Weslaco in 2004. Isolate F1 produced the most aflatoxin, followed by isolate I5 and isolate AF3357 (1774.19 ng g<sup>-1</sup>, 1681.71 ng g<sup>-1</sup> and 1621.81 ng g<sup>-1</sup>, respectively) (Figure 5-11).

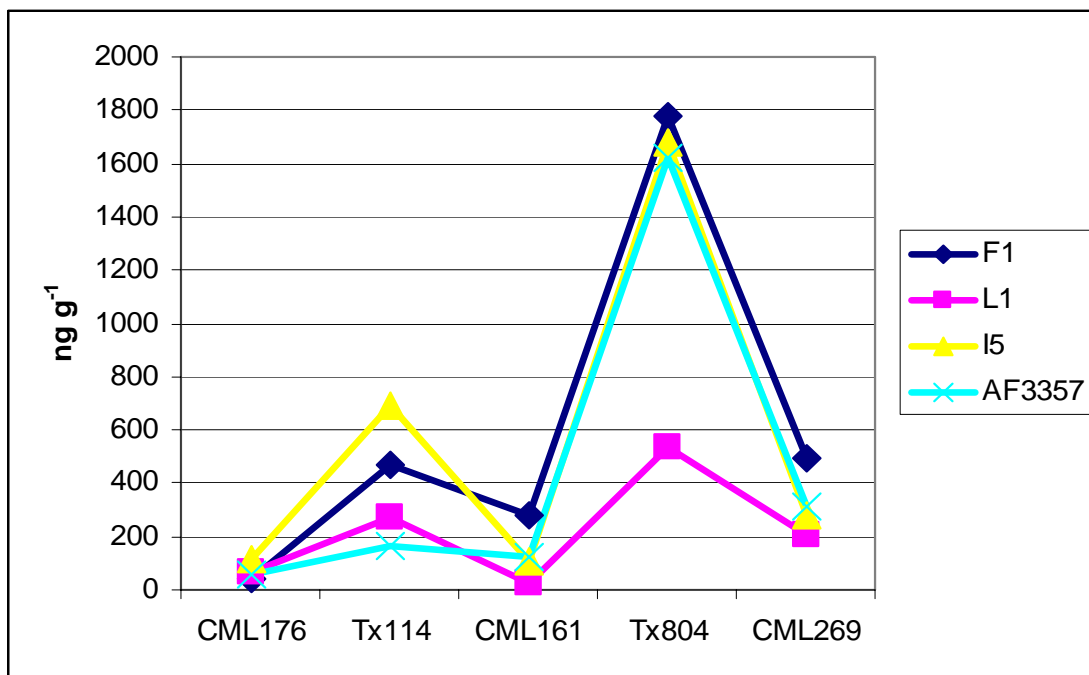


Figure 5-11. Aflatoxin accumulation per *A. flavus* isolate in inbreds at Weslaco in 2004

Analysis across environments in 2004 detected significant differences for environment, genotype, isolate and environment by isolate interaction (Table 5-9). Genotype by environment interaction, isolate by environment and environment by genotype by isolate interactions were non significant (Table 5-9).

Table 5-9. Analysis of variance for aflatoxin concentration in inbred trial across two locations in 2004

Source	DF	MS
Env	1	5.604***
Rep(Env)	6	0.482
Genotype	4	5.125***
Isolate	3	1.040*
G*I	12	0.273
Env*G	4	1.003**
Env*I	3	0.197
Env*G*I	12	0.241
Error	112	0.227

\*\* and \*\*\* Significant at 0.01  
and 0.001 Levels, respectively

\*\* and \*\*\* Significant at .01 and .001 levels respectively

DF: degrees of freedom. MS: Mean square

† Sources of Variation in ANOVA: Env=environment, rep(Env)=Reps within environments, G=genotypes, I=isolates, G\*I=genotype by isolate interaction, Env\*G=environment by genotype interaction, Env\*I=environment by isolate interaction, Env\*G\*I=environment by genotype by isolate interaction.

Isolates NRRL3357 and I5 produced the most aflatoxin in line Tx804 during 2004 (1894.52 ng g<sup>-1</sup> and 1852.99 ng g<sup>-1</sup>, respectively) (Figure 5-12).

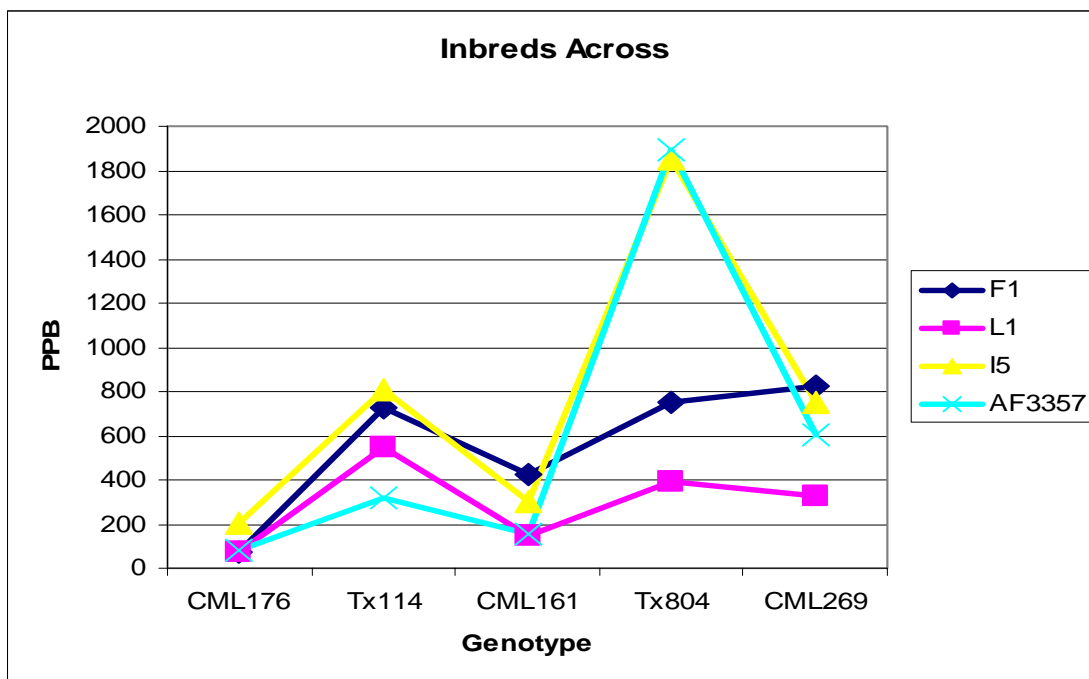


Figure 5-12. Aflatoxin accumulation per *A. flavus* isolate in inbred lines *per se* across locations in 2004

#### *Inbred per se Trial 2005*

No significant differences ( $P < .05$ ) were detected for replications at College Station or Weslaco (Table 5-10). Significant differences ( $P < .05$ ) were detected for genotypes at College Station and Weslaco (Table 5-10). No significant differences were detected for isolates at College Station but significant differences were detected at Weslaco (Table 5-10). No significant genotype by isolate interaction was detected for either College Station or Weslaco in 2005 (Table 5-10).

Table 5-10. Analysis of variance for aflatoxin accumulation in inbred trial at College Station and Weslaco in 2005.

Source	DF	CS <sup>†</sup>	WE
		MS	MS
Rep	3 <sup>‡</sup>	0.313	0.16
Genotype	4	4.989***	5.32***
Isolate	3	0.653	1.38*
Genotype*Isolate	12	0.339	0.50
Error	55	0.489	0.44

\* and \*\*\* Significant at .05 and .001 levels, respectfully

<sup>†</sup> Locations are CS=College Station and WE=Weslaco

<sup>‡</sup> College Station Rep degrees of freedom are 2 and error degrees of freedom are 34 due to the loss of the fourth rep of the test due to poor field conditions

Isolate I5 produced more aflatoxin (385.09 ng g<sup>-1</sup>) in College Station than the other isolates (Figure 5-13). Isolates F1 and I5 produced more aflatoxin (301.99 ng g<sup>-1</sup> and 293.80 ng g<sup>-1</sup>) than isolates L1 and AF3357 (Figure 5-13).

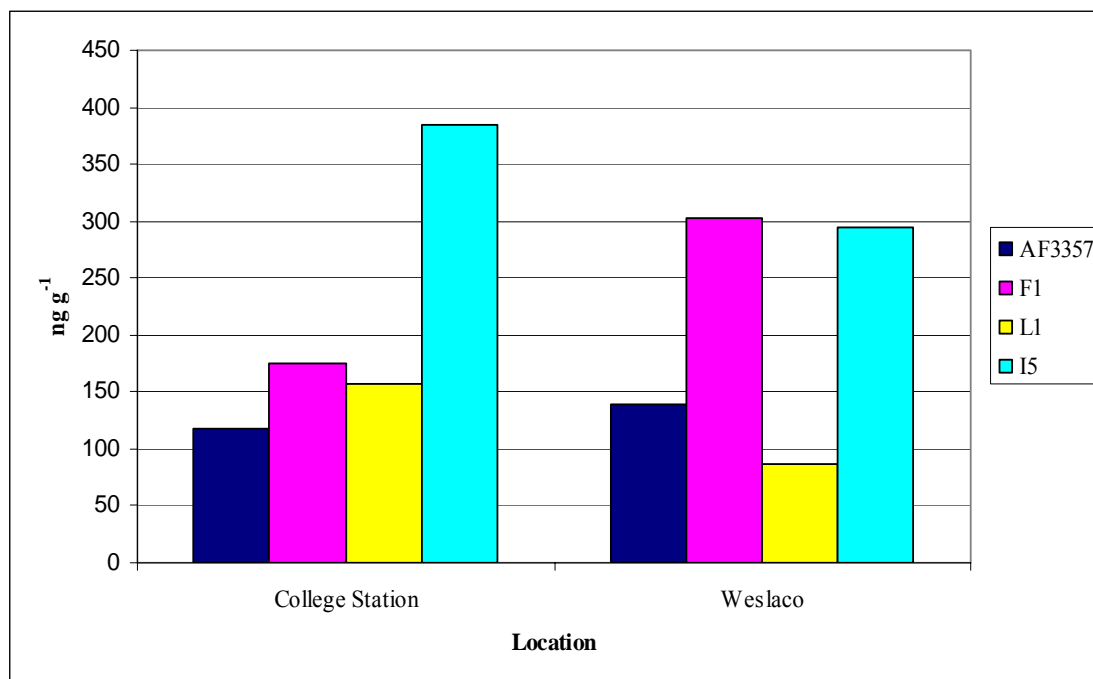


Figure 5-13. Mean aflatoxin accumulations by *A. flavus* isolates at College Station and Weslaco in 2005 in inbreds.

Isolates I5 and L1 produced the most aflatoxin in inbreds Tx804 and Tx114 (2393.32 ng g<sup>-1</sup> and 1914.26 ng g<sup>-1</sup>, respectively) and inbred Tx114 (1382.51 ng g<sup>-1</sup> and 874.76 ng g<sup>-1</sup>, respectively) (Figure 5-14).

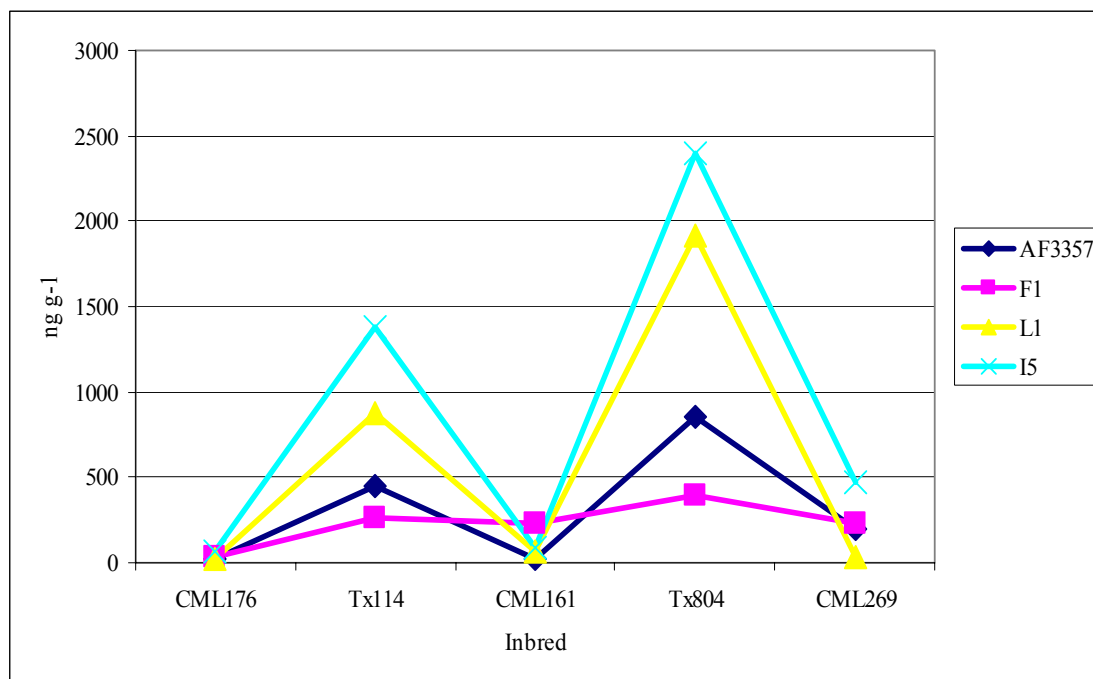


Figure 5-14. Aflatoxin accumulation per *A. flavus* isolate in inbreds at College Station in 2005

Isolates I5 and F1 produced the most aflatoxin in inbred Tx114 (2919.29 ng g<sup>-1</sup> and 1059.25 ng g<sup>-1</sup>, respectfully) and inbred Tx804 (891.25 ng g<sup>-1</sup> and 891.25 ng g<sup>-1</sup> respectively) (Figure 5-15).

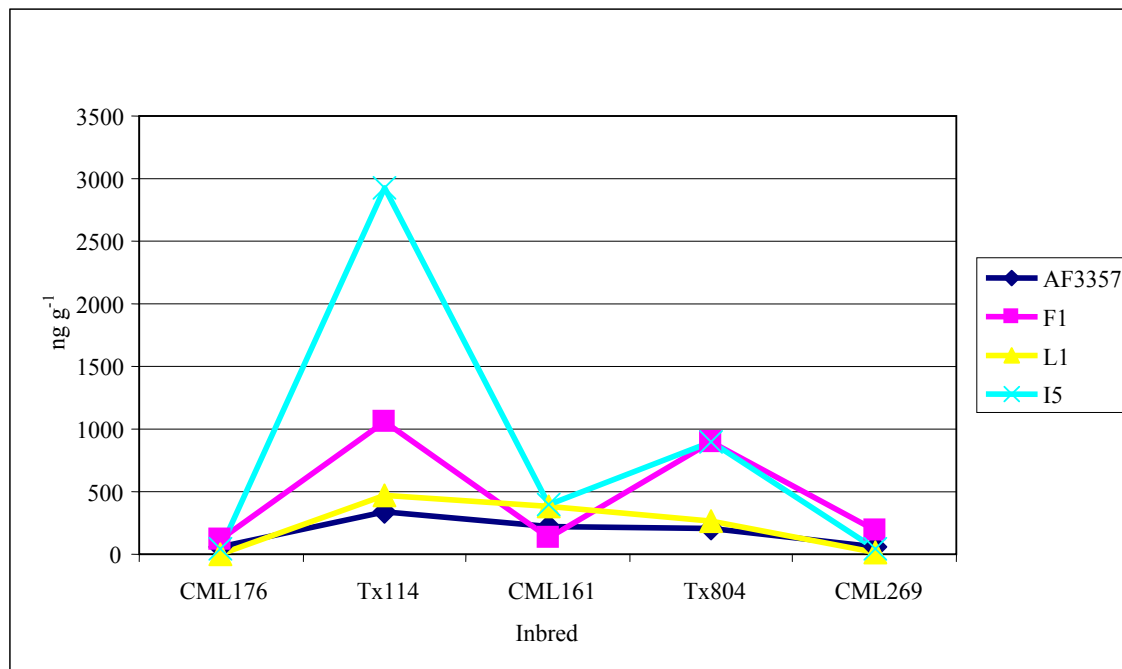


Figure 5-15. Aflatoxin accumulation per *A. flavus* isolate in inbreds at Weslaco in 2005

#### *Inbreds per se Across Environments 2005*

Significant differences were detected for genotypes across locations in 2005. All other sources of variation were significant (Table 5-11).



Table 5-11. Analysis of variance for aflatoxin concentration in inbred trial across locations in 2005

Source <sup>†</sup>	DF <sup>‡</sup>	MS
Env	1	0.011
Rep(Env)	5	0.223
G	4	8.903***
I	3	1.505*
G*I	12	0.369
Env*G	4	1.107
Env*I	3	0.383
Env*G*I	12	0.459
Error	89	0.459

\*\* and \*\*\* Significant at .01 and .001 levels respectively

<sup>†</sup> Sources of Variation in ANOVA: Env=environment, rep(Env)=Reps within environments, G=genotypes, I=isolates, G\*I=genotype by isolate interaction, Env\*G=environment by genotype interaction, Env\*I=environment by isolate interaction, Env\*G\*I=environment by genotype by isolate interaction.

<sup>‡</sup>DF: degrees of freedom. MSE: Mean square error

Isolate I5 accumulated the most aflatoxin in inbred Tx114 (2008.96 ng g<sup>-1</sup>) and Tx804 (1460.50 ng g<sup>-1</sup>) across environments (Figure 5-16).

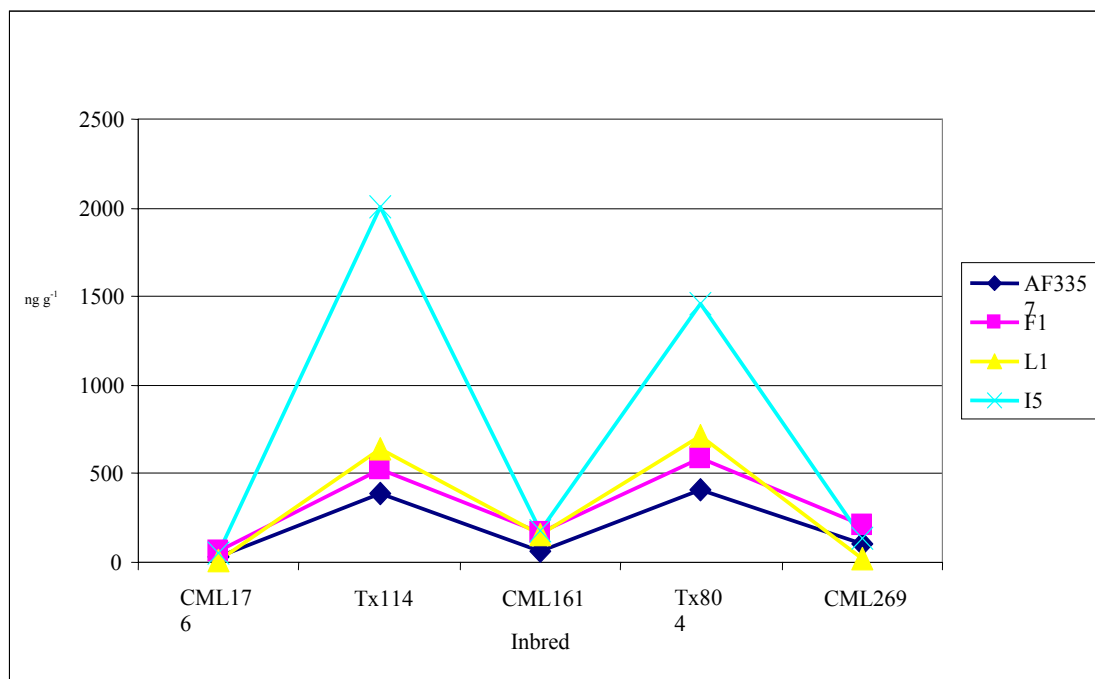


Figure 5-16. Aflatoxin accumulation per *A. flavus* isolate in inbreds across locations in 2005

#### *Inbreds per se Across Years 2004 and 2005*

Significant differences were detected for environment, genotype, isolate and environment by genotype interaction (Table 5-12). No significant differences were detected for replications (environment), genotype by isolate interaction, environment by isolate, and environment by genotype by isolate interactions (Table 5-12).

Table 5-12. Analysis of variance for aflatoxin concentration in inbred trial across years 2004 and 2005

Source <sup>†</sup>	DF <sup>‡</sup>	MS
Model	90	1.208***
Env	3	3.288***
rep(Env)	11	0.361
Genotype	4	12.851***
Isolate	3	2.509***
G*I	12	0.286
Env*G	12	1.347***
Env*I	9	0.359
Env*G*I	36	0.360
Error	201	0.344

\*\* and \*\*\* Significant at .01 and .001 levels respectively

† Sources of Variation in ANOVA: Env=environment, rep(Env)=Reps within environments, G=genotypes, I=isolates, G\*I=genotype by isolate interaction, Env\*G=environment by genotype interaction, Env\*I=environment by isolate interaction, Env\*G\*I=environment by genotype by isolate interaction.

‡DF: degrees of freedom. MSE: Mean square error

Across years and locations in inbreds *per se*, isolate I5 produced the most aflatoxin (506.12 ng g<sup>-1</sup>) (Figure 5-17). The isolate commonly used in field inoculations, NRRL3357, accumulated the lowest aflatoxin 202.38 ng g<sup>-1</sup> across environments, (Figure 5-17).

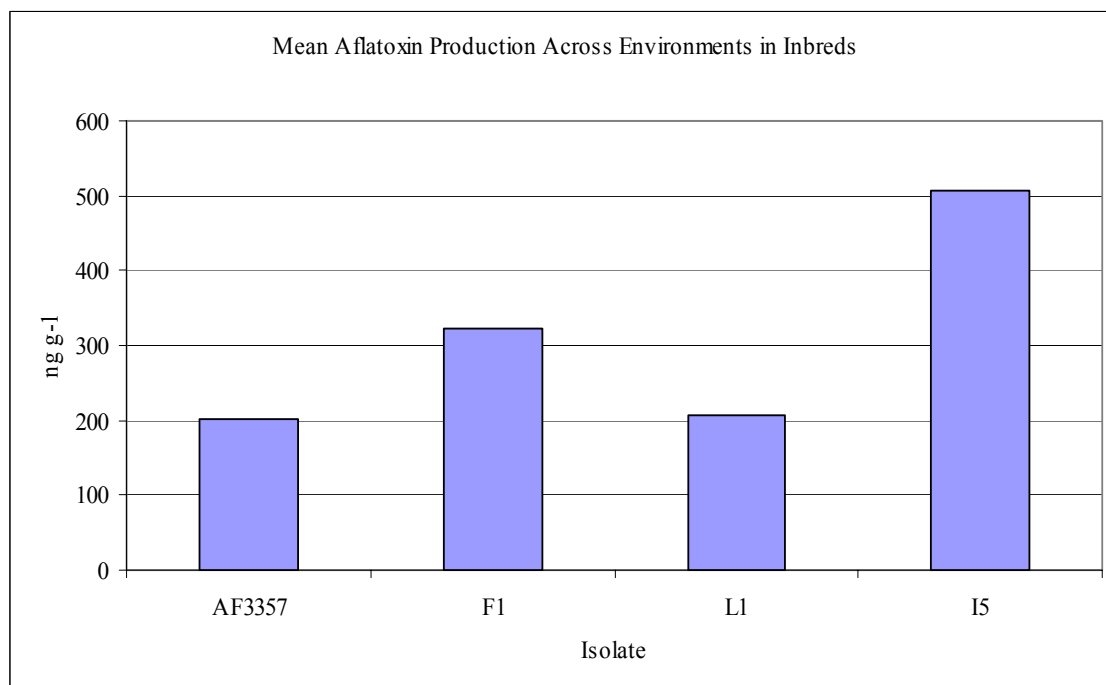


Figure 5-17. Means of aflatoxin accumulation by *A. flavus* isolate in inbred trial at two locations and two years

### Discussion and Conclusions

Differences exist in levels of aflatoxin produced by the different isolates (Mayfield et al., 2004). Isolate NRRL3357 did not cause the highest level of aflatoxin production in 2004 and 2005 either in hybrids or inbreds. Although NRRL3357 did have high levels of accumulation in some genotypes and years, this happened for those genotypes more susceptible to aflatoxin accumulation, e.g. hybrid SR470, and inbreds Tx804 and Tx114 and the year may have been the ideal year for aflatoxin production by this isolate.

Differences in aflatoxin accumulation occurred in hybrids and inbreds and across environments. No significant interaction was detected between genotypes and isolate in

either inbreds or hybrids, in either location, across locations with in a year or across years.

Isolate I5 and F1 produced more aflatoxin than NRRL3357. This does not insinuate that NRRL3357 is not a good isolate to use to screen for aflatoxin susceptibility. This isolate is discriminatory and produces differences between genotypes applied, which is what is needed for screening purposes. However, other isolates could serve the same purpose even better (isolates F1 and I5).

Significant differences in environment by isolate interaction were detected in 2004 and 2005 and across environment analysis in hybrids. One isolate of *Aspergillus flavus* may be used in screening for resistance, however; results may be limited in years and locations which are unfavorable for that isolate (Mayfield et al., 2005). Therefore, the use of a cocktail of *A. flavus* isolates known to produce aflatoxin may be necessary, in order to minimize potential effects of environment on the isolates, i.e. conditions which favor one isolates potential to produce aflatoxin under certain environmental conditions over another isolates potential..

## CHAPTER VI

### SUMMARY

Aflatoxin contamination of maize is a chronic and yearly problem in southern maize producing areas in the United States. Genetic host plant resistance has been determined to be the best solution for resistance to aflatoxin accumulations in maize (Munkvold, 2003; Moreno and Kang, 1999).

The maize breeding program at Texas A&M University has been evaluating breeding lines and germplasm for lowered susceptibility to preharvest aflatoxin accumulations in maize. Screening inbred lines *per se* and in hybrid combinations allows the selection of lines which have properties that reduce aflatoxin contamination. Hence, trials were conducted to evaluate breeding lines and hybrids in multiple locations and years. These trials were layout as alpha lattice field experimental designs with a variable number of replications from three to nine. The use of higher number of replications was utilized in anticipation of the variability of aflatoxin accumulation with in field experiments. All trials were inoculated to ensure uniform exposure of genotypes to the pathogen. All trials were either inoculated utilizing the non-wounding silk channel method (Zummo and Scott, 1989) or by utilizing the colonized kernel method (Odyssey, 2000). Colonized kernels were distributed as the first plots began to silk, in order for the inoculum to increase in the field and naturally inoculate the ears. This method worked well for inoculating large numbers of plots at one time, but had detrimental properties when high rains or irrigation came shortly after inoculation.

Betran et al. (2006b) showed strong correlations of aflatoxin and secondary traits including kernel texture and kernel integrity. Variation for these traits among genotypes was apparent in the each trial. This variation can facilitate the selection of lines with favorable expression of associated traits. Several lines such as Tx772 and derived lines from CIMMYT Population 69 are flinty, orange and show good husk cover. Wicklow et al. (1998) showed that a high level of  $\beta$ -carotene in the maize endosperm was a potential inhibitor of aflatoxin production.

The traits correlated with reduced aflatoxin accumulation in maize typically are not found in Midwest Corn Belt types of maize. These traits must be brought in from exotic sources (Betran et al., 2006a, 2006b). Many of the exotic sources used by the Texas A&M University breeding program are coming from tropical and subtropical sources from Central and South America (Betran et al., 2006b).

Inbred Tx772 *per se* and in hybrid combinations had lower aflatoxin accumulations in trials from 1999 to 2004. In *per se* trials, Tx772 accumulated less aflatoxin in all trials except one in year 2001. The Tx772 hybrids had a variable response for aflatoxin accumulation, ranging from the lowest in some tests to the highest in others. This illustrates the heavy environmental influence as well as the benefits of finding the right hybrid combination. Other inbred lines in *per se* trials or evaluated as hybrids were variable in aflatoxin accumulations. Inbreds CML285, CML288, CML323, CML325 CML326 and CML338 performed well in hybrid combinations for low aflatoxin accumulations. Inbreds evaluated *per se* that accumulated lower aflatoxin

concentrations include CML289, CML294 and CML323. CIMMYT inbreds performed less consistently across years in *per se* evaluations than in hybrids.

Traits such as husk cover, husk tightness, grain texture and kernel integrity had a positive response as secondary traits that reduce susceptibility to aflatoxin accumulation in maize; however, other unknown factors are still present which must be overcome for complete resistance. Selecting for these morphological, easily estimated, traits in early generation lines can enhance genetic improvement for aflatoxin resistance.

Inbred lines derived from crosses among lines previously evaluated for aflatoxin accumulation tended to show variability in reaction to aflatoxin accumulation. Lines derived from inbreds Tx772 and CML326 crossed with LH195 showed sufficient variability with high and low ranks depending on the location and year.

Aflatoxin production depends on three general factors: environment, host plant and pathogen. Environmental conditions favorable for aflatoxin production are generally hot, dry weather where the plant has stressed. Cultural practices that can reduce aflatoxin are proper planting time, recommended fertility regimen, supplemental irrigation if available and timely harvest (Jones, 1987). The second factor is the response of different maize genotypes to fungi colonization and aflatoxin production. Phenotypic and genotypic significant variation was observed in both inbreds and hybrids for aflatoxin concentration under inoculation as well as for other related agronomic traits. The variation present among genotypes can be exploited to develop less susceptible hybrids. The third factor is the pathogen and presence of toxigenic strain that produce aflatoxin. *Asperillus flavus* is variable in its natural environment. The



variability includes level of toxigenicity, from atoxigenic to highly toxigenic (Coty, 1997). In field trials, differences in aflatoxin production between toxigenic isolates exist. However, an isolate's capability to produce aflatoxin may be dependent upon the environmental conditions present at a particular location or year. No interaction between isolates and genotypes were observed. However, the use of a mixture of local *A. flavus* isolates may be recommended for more consistent and reliable aflatoxin production.

The multilocation and multiyear testing for aflatoxin testing presented here supports the need for multi environment evaluation to identify the most consistent resistant genotypes. Naidoo et al. (2002) also concluded that multilocation testing for aflatoxin accumulation was necessary. In order to increase locations tested, researchers have pooled their resources and began a regional aflatoxin test (SERAT) that has been conducted in 2004-2005. The SERAT tests were conducted at 6 and 9 locations across the south and in Illinois (Moore et al., 2004, Clements et al., 2005). This method of testing has increased testing capabilities for each program to evaluate their most resistant genotypes over more locations and evaluate genotypes under diverse conditions, inoculating techniques, and diverse isolates used as inoculum.

Progress has been made for breeding and selecting for aflatoxin resistance in maize at Texas A&M. Exotic inbred lines and populations have provided new alleles for traits for reduced aflatoxin accumulation. Introgression of exotic germplasm into locally adapted germplasm has improved agronomic characteristics such as husk cover, husk tightness and kernel type for use in the Southern U.S. and brought sources for lowered resistance to aflatoxin accumulation.

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## APPENDIX A

Means for aflatoxin, husk cover, grain texture and grain yield in inbred trial at College Station, TX in 2000.

Pedigree	AF <sup>†</sup>	HC	TXT	GY
	ng g <sup>-1</sup>	1 to 5	1 to 5	1 to 5
Tx772	25.7 d <sup>‡</sup>	1.8	1.1	2.9
Mp715	112.2 c	1.3	1.4	2.1
Tx601y	234.4 b,c	1.3	2.8	1.5
CML285	446.7 a,b	1.0	2.6	1.1
FR2128	501.2 a,b	2.0	2.0	2.5
MP420	707.9 a,b	1.3	2.1	2.8
MAS gk	1000.0 a	2.0	2.6	3.4
CML 326	1258.9 a	1.0	1.8	4.3
Mean	535.9	1.4	2.1	2.6
LSD <sup>§</sup>		0.0	0.6	0.0
Sig	***	**	***	***
C.V.% ¶	17.0	25.3	17.6	31.8

\*\* and \*\*\* Significant at .01 and .001 levels, respectively

† Traits are: AF: antilogarithmic transformation of data, HC: Husk Cover (1=long to 5=short), TXT: Grain Texture (1=flint to 5=dent), GY: visual rating for grain yield (1=high grain yield to 5=low grain yield)

‡ Mean separations determined using the logarithmic transformation of the data

§ Fisher's least significant difference

¶ C.V. %: Coefficient of Variation

## APPENDIX B

Means for aflatoxin, husk cover, grain texture, ear aspect, grain yield, insect damage and aflatoxin rating in inbred trial at Weslaco, TX in 2001

Pedigree	AF <sup>†</sup>	HC	TXT	EA	GY	ID	AFR
	ng g <sup>-1</sup>	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5	1 to 5
CML289	407.38 h <sup>‡</sup>	1.25	2.50	3.63	3.50	3.50	3.00
Tx601y	429.04 g,h	1.75	2.63	5.00	5.00	.	.
Tx732	441.57 g,h	2.50	3.38	1.75	1.38	2.88	2.88
Tx770	540.13 f-h	2.63	2.75	2.38	1.88	3.25	3.13
CML294	676.08 e-h	2.38	1.75	4.25	4.13	3.88	3.63
CML 323	724.44 d-h	3.13	1.25	2.75	3.00	2.50	2.63
CML288	966.05 c-g <sup>‡</sup>	1.00	1.50	2.00	2.38	2.63	3.00
B104	1122.02 b-f	1.38	2.38	2.88	2.75	3.75	3.13
NC300	1209.21 b-f	2.00	2.13	3.00	2.63	3.50	2.88
CML338	1453.78 a-e	1.13	2.00	2.75	2.75	2.75	2.75
FR2128	1612.50 a-d	1.63	1.63	1.63	1.75	2.00	2.63
Tx714	1757.92 a-c	2.00	2.25	2.50	1.88	2.75	3.00
B97	2053.53 a-c	2.63	4.63	4.63	3.13	4.25	4.75
A633	2317.39 a,b	1.88	1.25	3.00	3.13	3.38	3.00
CML285	2754.23 a	1.38	2.25	1.38	1.13	1.88	2.50
Mean	1231.02	1.91	85.03	2.28	3.06	2.90	3.06
LSD <sup>§</sup>	.	1.12	0.79	0.88	0.70	1.03	1.35
Sig.	***	**	***	***	***	**	**
C.V. % <sup>¶</sup>	8.31	41.26	24.31	21.14	18.30	23.53	30.84

\*\* and \*\*\* Significant at .01 and .001 levels, respectively

<sup>†</sup>Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint to 5=dent), EA: Ear Aspect (1=good ear aspect to 5=bad ear aspect), GY: visual rating for grain yield (1=high grain yield to 5=low grain yield), ID: visual rating for insect damage to kernels on ear (1=few ears damaged to 5=most ears damaged with channeling), AFR: visual rating for *Aspergillus flavus* Colonization (1= no colonization to 5=all ears colonized)

<sup>‡</sup> Mean separations determined using the logarithmic transformation of the data

<sup>§</sup> Fisher's least significant difference

<sup>¶</sup> C.V. %: Coefficient of Variation



## APPENDIX C

Means for aflatoxin, grain texture, appearance and grain yield in inbred trial at College Station, TX in 2002.

Pedigree	Aflatoxin <sup>†</sup>	TXT	APP	GY
	ng g <sup>-1</sup>	1-5	1-5	Mg ha <sup>-1</sup>
Tx601y	1.0 e <sup>‡</sup>	2.11	4.54	0.13
NC300	1.3 e	2.05	1.04	1.40
A633	1.7 e	0.98	2.17	0.75
CML285	1.7 e	1.99	2.81	0.90
CML288	1.8 e	0.98	2.53	0.90
CML338	4.2 d,e	2.13	3.19	0.59
B104	8.5 b-e	1.66	2.59	0.88
FR2128	8.7 b-e	1.75	1.96	1.43
Tx770	23.0 ,c,d	2.23	2.73	1.25
CML 325	24.7 b-d	2.29	2.56	0.80
Tx732	28.4 b-d	3.02	2.44	1.37
Tx714	34.7 b-d <sup>‡</sup>	2.11	2.99	1.01
Tx760	74.5 a-c	4.93	4.10	1.04
CML 323	84.3 a,b	1.01	2.73	0.75
B97	543.9 a	4.50	3.50	1.00
Mean	56.2	2.25	2.79	0.95
LSD <sup>§</sup>		0.58	1.12	0.45
Sig	***	***	***	***
C.V. % <sup>¶</sup>	74.0	18.77	28.63	33.75

\*\*\* Significant .001 levels

<sup>†</sup>Traits are: AF: antilogarithmic transformation of data, TXT: Grain Texture (1=flint to 5=dent), APP: Plant Appearance (1=good plant appearance to 5=poor plant appearance)

<sup>‡</sup> Mean separations determined using the logarithmic transformation of the data

<sup>§</sup> Fisher's least significant difference

<sup>¶</sup> C.V. %: Coefficient of Variation

## APPENDIX D

Means for aflatoxin, anthesis date, silking date, anthesis silking interval, kernel texture, insect damage and desirability in inbred trial at Weslaco, TX in 2002.

Pedigree	Aflatoxin <sup>†</sup>	MF	FF	ASI	TXT	ID	DES
	ng g <sup>-1</sup>	d	d	d	1 to 5	1 to 5	1 to 5
Tx732	5.6 f <sup>‡</sup>	79.75	82.00	-2.25	3.00	3.38	2.49
Tx714	13.3 e,f	79.00	82.00	-3.00	2.75	3.50	3.56
CML285	31.9 d-f	84.50	85.50	-1.00	1.94	3.04	4.49
B104	35.3 d-f	84.00	85.50	-1.50	2.25	2.75	3.05
FR2128	66.3 c-e	81.25	82.00	-0.75	1.75	3.50	3.34
Tx760	87.4 b-e	80.50	82.00	-1.50	4.13	4.75	4.61
CML288	107.2 b-e	86.00	87.00	-1.00	1.00	2.25	3.62
CML 325	118.9 a	76.00	79.75	-3.75	1.50	2.25	2.79
NC300	196.6 a-d	82.00	82.00	0.00	2.00	2.25	2.30
A633	254.0 a-d	79.00	82.00	-3.00	1.00	2.63	3.31
CML 323	265.2 a-d	84.50	83.00	1.50	1.00	3.25	2.32
CML338	518.8 a-c	79.00	82.00	-3.00	1.00	2.25	3.46
B97	611.3 a,b	80.50	82.00	-1.50	4.13	4.25	3.04
Tx770	1490.2 a	81.25	82.50	-1.25	2.88	3.50	3.44
Tx601y	.	86.75	89.50	-2.75	.	.	.
Mean	324.9	81.60	83.25	-1.65	2.17	3.11	3.26
LSD <sup>§</sup>	.	1.53	1.36	1.74	0.62	0.76	0.88
Sig	***	***	***	***	***	***	***
C.V. % <sup>¶</sup>	33.3	1.33	1.16	-76.72	20.16	17.34	20.07

\*\*\* Significant .001 levels

<sup>†</sup>Traits are: AF: antilogarithmic transformation of data, MF: Days from planting to anthesis, FF: days from planting to silking, ASI: Anthesis silking interval (MF-FF), TXT: Grain Texture (1=flint to 5=dent), ID: Insect damage (1=no damage or channeling to 5=heavy damage or channeling), DES: Desirability (1=desirable plant type to 5=non desirable plant type).

<sup>‡</sup> Mean separations determined using the logarithmic transformation of the data

<sup>§</sup> Fisher's least significant difference

<sup>¶</sup> C.V. %: Coefficient of Variation

## APPENDIX E

Means for aflatoxin, anthesis date, silking date, anthesis silking interval, kernel texture, insect damage and desirability in inbred trial at Weslaco, TX in 2003.

Pedigree	AF <sup>†</sup>	FF	MF	ASI	TXT	KI	DES
	ng g <sup>-1</sup>	d	d	d	1 to 5	1 to 5	1 to 5
Tx772	9.6 g <sup>‡</sup>	71.75	69.00	2.74	1.38	2.00	1.50
Pop. 69 -B-B-B4-7-B-B	9.6 g	72.50	70.00	2.87	1.50	1.75	2.38
Pop. 69 -B-B-B2-2-B-B	15.4 f,g	71.00	67.00	4.16	1.50	1.25	1.75
CML285	17.3 e-g	77.50	75.50	2.14	2.51	3.51	3.54
Pop. 69 -B-B-B3-6-B-B	21.9 e-g	72.50	69.00	3.15	1.50	1.88	1.75
NC300	37.5 d-g	74.00	71.00	3.19	2.00	1.63	1.63
Pop. 69 -B-B-B4-11-B-B	47.2 d-f	72.50	69.00	3.35	1.50	1.63	1.88
Pop. 69 -B-B-B5-7-B-B	48.8 d-f	70.75	69.25	1.40	1.50	1.75	1.88
Pop. 69 -B-B-B3-5-B-B	53.6 d-f	71.75	70.50	1.00	1.50	1.25	1.50
CML338	74.2 d,e	71.75	68.50	3.88	1.63	1.75	1.88
Tx770	144.7 c,d	74.00	71.00	2.93	3.50	2.88	2.38
FR2128	364.1 b,c	74.00	71.00	3.24	1.88	2.25	1.75
B104	368.2 b,c	74.50	72.50	1.31	2.63	2.88	2.25
CML288	477.7 b,c	67.00	64.00	2.95	.	.	.
Tx732	584.5 a-c	72.50	70.00	2.92	3.50	3.50	2.00
CML 323	628.5 a-c	71.75	72.00	-0.20	1.63	2.63	2.63
Tx714	820.7 a,b	72.00	70.00	2.03	2.25	3.50	3.38
CML 325	936.5 a,b	75.50	75.50	0.27	1.50	3.38	3.13
B97	2205.6 a	71.00	69.75	0.63	4.00	4.00	3.50
Tx601y	.	78.00	76.00	1.79	1.94	1.55	3.01
Mean	361.3	72.81	70.53	2.29	2.07	2.38	2.25
LSD	.	2.21	2.83	1.9	0.37	0.80	0.77
Sig	***	***	***	***	***	***	***
CV	23.5	2.16	2.85	62.12	12.60	23.97	24.20

\*\*\* Significant .001 levels

<sup>†</sup>Traits are: AF: antilogarithmic transformation of data, FF: days from planting to silking, MF: Days from planting to anthesis, ASI: Anthesis silking interval (MF-FF), TXT: Grain Texture (1=flint to 5=dent), KI: kernel integrity (1=no kernels broken on ear to 5=majority of kernels broken on ear), DES: Desirability (1=desirable plant type to 5=non desirable plant type).

<sup>‡</sup> Mean separations determined using the logarithmic transformation of the data

<sup>§</sup> Fisher's least significant difference

<sup>¶</sup> C.V. %: Coefficient of Variation

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